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THE JOURNAL OF AGRICULTURAL SCIENCE

EDITED FOR THE PLANT BREEDING AND ANIMAL NUTRITION RESEARCH INSTITUTES AT CAMBRIDGE,
AND THE ROTHAMSTED RESEARCH INSTITUTES BY

PROFESSOR R. H. BIFFEN, M.A., F.R.S., Cambridge
SIR A. D. HALL, K.C.B., M.A., LL.D., F.R.S., Ministry of Agriculture, London
F. H. A. MARSHALL, Sc.D., F.R.S., Cambridge
SIR E. J. RUSSELL, D.Sc., F.R.S., Rothamsted Experimental Station, Harpenden
PROFESSOR T. B. WOOD, C.B.E., M.A., F.I.C., F.R.S., Cambridge

IN CONSULTATION WITH

B. C. ASTON, Department of Agriculture, Wellington, New Zealand
DR C. A. BARBER, C.I.E., School of Agriculture, Cambridge
PROFESSOR B. T. P. BARKER, M.A., National Fruit and Cider Institute, Long Ashton, Bristol
W. BATESON, M.A., F.R.S., John Innes Horticultural Institute, Merton, Surrey
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DR A. C. TRUE, Department of Agriculture, Washington, D.C., U.S.A.
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DR H. J. WHEELER, American Agricultural Chemical Co., Boston, Mass., U.S.A.

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CONTENTS

PART 1 (JANUARY 1922)

	PAGE
NEWTON, ROBERT. A comparative study of winter wheat varieties with especial reference to winter-killing . . .	1
GOODEY, T. On the susceptibility of clover and some other legumes to stem-disease caused by the eelworm, <i>Tylenchus dipsaci</i> , syn. <i>devastatrix</i> , Kühn. (With Plate I) . . .	20
SALAMAN, R. N. and LESLEY, J. W. Genetic studies in potatoes; sterility. (With Plate II)	31
CROWTHER, CHARLES and WOODMAN, HERBERT ERNEST. A study of nitrogen metabolism in the dairy cow . . .	40
ARMSTRONG, S. F. The Mendelian inheritance of susceptibility and resistance to yellow rust (<i>Puccinia glumarum</i> , Erikss. et Henn.) in wheat	57
WOODMAN, HERBERT ERNEST and HAMMOND, JOHN. Note on the composition of a fluid obtained from the udders of virgin heifers	97

PART 2 (APRIL 1922)

MURRAY, ALAN J. The chemical composition of animal bodies. (With 1 Text-figure)	103
TAYLOR, WILLIAM and HUSBAND, ALFRED D. The effect on the percentage composition of the milk of (<i>a</i>) variations in the daily volume and (<i>b</i>) variations in the nature of the diet. (With 4 Text-figures)	111
TOCHER, J. F. The citric solubility of mineral phosphates. (With 8 Diagrams)	125
WOODMAN, HERBERT ERNEST. Comparative determinations of the digestibility and metabolisable energy of green oats and tares, oat and tare hay and oat and tare silage	144
WHITTLES, C. L. A note on the classification of soils on the basis of mechanical analyses. (With 11 Text-figures)	166
SALAMAN, REDCLIFFE N. The Influence of size and character of seed on the yield of potatoes. (With 4 Text-figures)	182
ENGLEDOW, F. L. and SHELTON, J. P. An investigation upon certain metrical attributes of wheat plants	197

PART 3 (JULY 1922)

	PAGE
DEIGHTON, THOMAS. Some investigations on the electrical method of soil moisture determination. (With 6 Text-figures)	207
WOODMAN, HERBERT ERNEST. The chemistry of the strength of wheat flour	231
IVANOFF, E. I. On the use of artificial insemination for zootechnical purposes in Russia	244
CAPSTICK, J. W. and WOOD, T. B. The effect of change of temperature on the basal metabolism of swine. (With 4 Text-figures)	257
HORTON, E. and SALMON, E. S. The fungicidal properties of certain spray-fluids. III	269
COLLINS, S. H. and THOMAS, B. The sugars and albuminoids of oat straw	280
ROBINSON, GILBERT WOODING. Note on the mechanical analysis of humus soils	287
JONES, S. G. A bacterial disease of turnip (<i>Brassica napus</i>). (With Plate III)	292
ROBINSON, GILBERT WOODING. A new method for the mechanical analysis of soils and other dispersions. (With 4 Text-figures)	306

PART 4 (OCTOBER 1922)

AMOS, ARTHUR and WILLIAMS, GWILYM. Temperature and other factors affecting the quality of silage	323
AMOS, ARTHUR and WOODMAN, HERBERT ERNEST. An investigation into the changes which occur during the ensilage of oats and tares	337
COMBER, NORMAN M. The availability of mineral plant food. A modification of the present hypothesis	363
COMBER, NORMAN M. A modified test for sour soils	370
COMBER, NORMAN M. The flocculation of soils. III	372
HAMMOND, JOHN. On the relative growth and development of various breeds and crosses of pigs. (With 5 Text-figures)	387

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A COMPARATIVE STUDY OF WINTER WHEAT VARIETIES WITH ESPECIAL REFERENCE TO WINTER-KILLING¹.

BY ROBERT NEWTON, M.S., B.S.A.

University of Alberta, Edmonton.

INTRODUCTORY.

WINTER wheat, where it can be safely grown, usually outyields spring varieties quite markedly, but unfortunately it is much more restricted in distribution, due to its liability to winter-killing. Much progress has been made with this and other crops in breeding for cold resistance by empirical methods. It would appear, however, that greater and more certain progress would be made if the nature of cold resistance in plants were well understood. This subject has long been of interest to physiologists, and in its practical applications is of widest importance. The northern limit of profitable growth of our staple crops marks also the limit of profitable exploitation of our agricultural lands. The southern farmer has likewise to meet the problems of the frost-killing of fruit buds and flowers and the more tender winter cereals.

HISTORICAL.

The progress of our knowledge of the nature of cold resistance and frost effects has been reviewed quite fully at different times by Abbe (1), Blackman (5), Chandler (6) and others. Of the earlier investigations it will be sufficient to note here in their order the most significant.

The theory put forward in 1737 by Duhamel and Buffon (11) that death from cold was due to rupturing of the cell walls by expansion on ice formation, is of historical interest. It was almost a century later that Goeppert (13) found ice formation to occur in the intercellular spaces. Saebs (38) showed this to be the usual occurrence, and developed the view, now generally considered erroneous, that disorganisation took

¹ This study was carried on in the laboratory of Dr R. A. Gortner, Chief of the Division of Agricultural Biochemistry, University of Minnesota, to whom grateful acknowledgement is made for kind help and direction. The work was aided by election to the Shevlin Fellowship.

place on thawing, and might be prevented by warming very slowly, allowing time for reabsorption of the water by the cell.

Later Müller-Thurgau⁽²⁹⁾ proved that this ice formation in the tissues was necessary for freezing to death, and concluded that death was due to the consequent desiccation of the protoplasm. This hypothesis received support from Matruchot and Molliard⁽²⁹⁾, who demonstrated the identity of the modifications in cell structure produced by frost, plasmolysis and desiccation. The work of Greeley⁽³⁷⁾ supplied similar evidence. Mez⁽³⁸⁾ opposed the theory of death by desiccation, since his investigations indicated that all solutes crystallise out at a temperature not lower than -6°C . He concluded that cold desiccation must therefore be complete at this temperature, and cannot explain injury to plants which resist much lower temperatures. He advanced instead the theory of a fatal minimum temperature for each plant.

Görke⁽¹⁴⁾ showed another important effect of the withdrawal of water, namely, the precipitation of certain proteins by the increasing concentration of the cell sap, aided by its increasing acidity on cooling. He also showed that the precipitation occurred at varying temperatures for plants of varying degrees of hardiness. This was ascribed by Schaffnit⁽¹⁰⁾ to the splitting in varying degrees during the hardening process of complex proteins into simpler, less readily precipitated forms.

Lidforss⁽²⁶⁾ found most hardy plants to have their starch reserves converted to sugar during the winter, and believed this an adaptation for cold resistance, the sugar having a protective action in preventing the precipitation of the proteins. Schaffnit⁽¹⁰⁾ tested the effect of adding sugars and various other substances to plant saps and to egg albumen solution, and was able to modify very greatly the precipitation by freezing. Equally striking results were secured by Maximov⁽³⁰⁾ in increasing the hardiness of sections of red cabbage and *Tradescantia* by freezing them in solutions of either organic or inorganic substances, provided these were non-toxic and had a low eutectic point.

RECENT PROGRESS.

Recent investigations have dealt mainly with the hypotheses noted above, extending them in several important respects. Attempts have also been made to determine the correlation of various physical, chemical, physiological and morphological characters with apparent frost hardiness.

Several workers have drawn attention to the possible importance of the fact that plant sap is contained in cells of capillary dimensions. D'Arsonval⁽³⁾ estimated the osmotic pressure in very small cells at

1000 atmospheres, and notes that by the application of increasing pressure the solidification point of water can be lowered indefinitely. In buds with small cells, dense tissues and meagre water content, Wiegand (49) found no ice at -18°C ., though in most buds it was present in large quantity. He concluded that the degree of cold necessary to cause the separation of ice is proportional to the force which holds the water in the tissues. Lewis and Tuttle (25) reported that living leaves of *Pyrola* wrapped around the bulb of a mercury thermometer undercooled to -32.1°C . before ice formation took place. On the other hand, it has been a universal observation since the time of Goepfert (13) that ice may form in the tissues without injury to hardy plants, so that undercooling is not of itself a sufficient explanation of hardiness.

The water content of tissues is related to structure, and it has been shown by several investigators (2, 4, 24, 37, 40, 43, 44) that dry matter content is directly correlated with hardiness. Sinz (44) and Beach and Allen (1) noted also the importance of structures resisting desiccation, while Pantanelli (35) found injury from frost to be always proportionate to loss of water from the tissues, even when freezing was done in a saturated atmosphere.

Between the concentration of the cell sap and winter hardiness, Ohlweiler (34) and Chandler (6) found a direct relationship; Salmon and Fleming (39), working with winter cereals, found none. Pantanelli (35) partly reconciled the conflicting evidence by reporting a relationship in some crops and none in others, including wheat. Probably the sap of all plants increases in concentration during the hardening process, but not necessarily in proportion to the degree of hardiness attained. However, the earlier evidence as to the importance of the accumulation in the sap of substances of a protective nature, especially sugars, has received further support. Gassner and Grimme (12) and Åkerman and Johansson (2) reported that hardy varieties of winter wheat and other grains were richer in sugar, the differences between varieties corresponding to differences in degree of hardiness. Pantanelli (35) found that sugar was rapidly used up during exposure to low temperatures, and that hardiness was related to the quantity of sugar retained by the plant. The association of sugar accumulation with hardening by cold has been pointed out again by Rosa (37) and Coville (7).

On the other hand, Harvey (19) found that cabbages acquired hardiness on five days' exposure to $+3^{\circ}\text{C}$., before any great change occurred in the carbohydrate equilibrium. He believes the principal effect of the hardening process to be a change in the constituents of the protoplasm,

as indicated by an increase in the amino acid content, and, on freezing the sap, by less precipitation of the proteins. He measured the increase in hydrogen-ion concentration of the sap on cooling, and was able to produce the same relative precipitation of proteins by adding equivalent quantities of acid. However, the ease of precipitation of proteins by freezing apparently cannot always be taken as an index of hardiness, since Chandler (6) was unable to find any difference in this respect between the sap of tender and hardy twigs of fruit trees. Again, it should perhaps be remarked that the fraction of the total proteins present in the expressed sap is rather small, and of this only 31.2 per cent. at most is reported by Harvey as precipitated by freezing the juice of unhardened cabbage. The extent to which this justifies a generalisation with regard to the probable behaviour of the proteins within the cell may be regarded as problematic.

There is some evidence that stability of the dormant condition may be an important protective adaptation. Lidforss (26) noted that a succession of warm days caused regeneration of starch from sugar, with an increase in susceptibility to cold. Chandler (6) found some varieties of peaches to have a longer rest period than others and to be started into growth more slowly by warm periods in the winter. Evidently varieties which can maintain continuous dormancy during the danger period must have a distinct advantage.

Our present concept of the causes of winter-killing may be briefly summarised. Without doubt, the ultimate cause of death by freezing must be the disorganisation of the protoplasm. Irreversible coagulation or precipitation of the colloidal protein constituents may be caused by increase in concentration of electrolytes in the cell sap on withdrawal of water, or by increase in acidity, or by both factors acting together. The critical minimum temperature necessary to bring this about must be profoundly modified by rate of cooling, especially if this be slow enough to give time for the hardening process, and by the presence of substances which protect the proteins from precipitation. Splitting of the proteins during hardening may be a protective adaptation. Since the fundamental feature of the disturbance produced by freezing is withdrawal of water from the cell, intracellular adaptations to resist desiccation must be of prime importance.

EXPERIMENTAL.

THE PROBLEM.

The present study seeks to establish a chemical or physico-chemical measurement of hardiness for winter wheat varieties. A number of varieties originated or selected by the Department of Plant Breeding of the University of Minnesota, and known to vary considerably in hardiness, were compared with reference to the physical constants of the cell sap, the content of amino nitrogen, water-soluble nitrogen and total nitrogen, and the content of sugars and starch. All material used was grown in field plots under normal conditions. Since it was desired to compare the varieties in the hardened condition rather than to study the hardening process, collections were not made until after the advent of freezing weather.

A preliminary study of physical constants was carried out with eight varieties. Subsequent study was confined to four of these, two hardy and two tender. One variety, Minhardi, was collected from two plots some distance apart, and these are reported separately as the effect of location was quite marked.

METHODS.

Collection of Samples. All the samples of one series were collected from the field the same afternoon, though with the exception of the first series the leaves were frozen solid when collected, so that changes due to vital activities would be very slight. The plants were growing in rows, which were carefully gone over for the removal of dead leaves before taking the samples. For the collection of November 12, 1920, it was necessary first to brush off a light covering of snow. As the leaves were cut, they were thrown on a wire screen for the removal of adhering bits of dirt and ice, then transferred at once to tight glass containers. Samples of approximately 100 grams were collected in duplicate, one lot for the study of physical constants, the other for analysis for nitrogen and carbohydrates. All samples were kept frozen until used.

Physical Constants. The depression of the freezing point of the first collection was determined by the thermoelectric method, the accuracy of which has been shown by White (17). The convenient arrangement of apparatus illustrated by Harvey (20, Fig. 1) was used. The leaves were packed into a section of thin-walled glass tubing 2 cm. long, in which they were held in place by a small rubber band, the thermocouple then being inserted in the centre. Undercooling seldom amounted to more

than 2° C. and was corrected for in the usual way. By this method duplicates often varied as much as 0.03° C., and in later collections it was abandoned in favour of the standard Beckmann method, by which it was always possible to obtain checks agreeing within 0.01° C.

The work of Dixon and Atkins (10), extended by Gortner, Lawrence and Harris (6), has shown the necessity of rendering the cell membranes permeable by freezing the tissue previous to sap extraction in order that a representative sample may be obtained. However, having regard to the observation of Harvey (19) that freezing permanently lowered the hydrogen-ion concentration of cabbage juice, and (21) that on the other hand a certain amount of dilution did not affect this value, the samples of the first collection were not frozen before expressing the sap, as it was desired to study particularly the relationship of this constant to hardness. But the following comparisons of sap expressed from duplicate samples with and without previous freezing indicated that for wheat at least Harvey's observation does not hold true.

Variety		Not frozen	Frozen
		pH	pH
Minhardi	...	6.580	6.376
Bullum	...	6.465	6.289
Padui	...	6.475	6.289

Therefore in later collections preliminary freezing of the tissues was carried out, and the expressed sap used for all constants studied.

The technique of Gortner and Harris (15) was followed in the main. The rubber-stoppered bottles containing the samples were packed in a slushy mixture of pulverised ice and salt in an earthenware jar, which fitted snugly inside a well-insulated "fireless cooker." In this condition the contents remained frozen solid until required for use, never in any case for less than 12 hours. For the freezing mixture, common salt was used at first, and later calcium chloride. To thaw the samples, the bottles were placed under running water, then rinsed with distilled water and wiped dry before opening. The leaves were folded in pieces of strong cotton previously boiled in three changes of distilled water and dried free from dust, and the sap expressed either in a hydraulic press under 400 atmospheres pressure, or in a large hand screw press with a small steel cup which permitted the application of heavy pressure. The parts of the press with which the juice came in contact were kept coated with a thin layer of paraffin wax.

The depression of the freezing point was first determined with a Beckmann apparatus. Then the conductivity was measured with a

Wheatstone bridge, using a Freas conductivity cell, and finally the hydrogen-ion concentration was determined by means of standard Leeds and Northrup potentiometric equipment. Both of the latter determinations were carried out at 25° C. in a constant temperature room. Usually the work on any particular sample was completed within an hour of expressing the juice.

Preparation of Samples for Analysis. As the samples for analysis were collected in the field they were placed directly in tared one-litre erlenmeyers with rubber stoppers. They were frozen when collected, and kept in that condition overnight. Immediately after thawing in the laboratory next day, samples for the determination of dry matter were weighed out; then in addition, sufficient material was removed to leave exactly 100 grams in each flask. To this was added 1.5 grams of pure precipitated calcium carbonate for the neutralisation of plant acids, and sufficient 95 per cent. alcohol to make the final concentration 80 per cent. after allowing for the dilution due to water in the leaves. The samples were then boiled half an hour under reflux condensers, and put away tightly stoppered until a convenient time for analysis. The procedure from thawing to boiling was carried out with the utmost expedition.

The advantage of using calcium carbonate as noted above has been discussed by Spoehr (45). Davis, Daish and Sawyer (9) have pointed out the necessity for rapid destruction of enzymes. In this connection the present study afforded opportunity for some observations of interest. An additional quantity of one variety was collected for experimentation in methods. Part of this was left five days in an ice chest, and was then put through the regular preparative and analytical procedure. In Table I the results are compared with those for the same material disposed of promptly after collection. When it is considered that the tissues were not crushed or injured to any appreciable degree, the effect of enzyme action even in such cold storage conditions is very striking.

Dry Matter. Triplicate samples of approximately 5 grams of green material were dried to constant weight in a vacuum oven at 98° C.

Total Nitrogen. The total nitrogen was determined by the Kjeldahl-Gunning-Arnold method, using the residues from the dry matter determinations.

Extraction of Sugars and Soluble Nitrogen. Large extractors of the Soxhlet type were made by drilling a small hole close to the bottom of a 750 c.c. wide-mouthed bottle, and fitting in a glass siphon tightly by making a ground glass joint or by wedging it with a collar of rubber tubing. The bottle was closed with a large rubber stopper, in which were

fitted two reflux condensers and a bent glass tube leading to the distilling flask below. The siphon also passed through the stopper of the distilling flask. The samples were transferred to one of these extractors and extracted with 80 per cent. alcohol on a steam bath for 30 hours, by which time the Molisch α -naphthol test on the alcohol in the extractor always became entirely negative. In transferring material from one container to another, the emptied container was thoroughly rinsed with hot alcohol.

The extract was concentrated in the apparatus illustrated by Van Slyke (16, Fig. 1) under a pressure of less than 30 mm., with the distilling flask in a water bath at a temperature of 40 to 50 C. When reduced to a volume of 75 to 100 c.c., about 200 c.c. of distilled water was added and the solution reconcentrated to get rid of the last traces of alcohol. This precaution was found necessary since the presence of alcohol affected the subsequent determination of amino nitrogen. The reconcentrated extract was then transferred to a 250 c.c. volumetric flask by filtering through a pad of cheesecloth in a small funnel, making it nearly to volume by several successive washings of the distilling flask with small portions of boiling water. A pad of cheesecloth four layers thick was found the most satisfactory filter for removing the solid particles of chlorophyll which separated out. All other materials tried clogged at once. The extract was then cooled to room temperature and made up to volume.

All volumetric flasks and pipettes used throughout the analyses were standardised in true cubic centimetres at 20° C.

Amino Nitrogen. The amino nitrogen was determined by the usual Van Slyke apparatus, using 10 c.c. portions of the extract, filtered for removal of fine particles which had escaped the cheesecloth filter. Since preliminary trials had given a somewhat higher yield when deamination was continued for 30 minutes instead of the usual five minutes, the former period was adopted. Of this time, shaking was done during the first minute and the last two minutes.

Water-Soluble Nitrogen. The total water-soluble nitrogen in the alcoholic extract was determined by the Kjeldahl-Gunning-Arnold method, using 10 c.c. portions of the concentrated extract, filtered as for amino nitrogen.

Clearing Extract for Sugar Analysis. After the portions required for nitrogen determinations had been removed, the remainder of the extract was cleared with dry powdered lead acetate. The dry defecation method, first proposed by Horne (23), has the advantage not only of largely

eliminating the error due to the volume of the precipitate, but also of obviating the necessity of making to volume a second time. The lead acetate was added in small quantities at a time, successive small portions of the extract being filtered off and tested for completeness of precipitation. The solution was then filtered through a dry filter paper and deleaded with a minimum quantity of powdered sodium oxalate, following the same technique as for clarification. A second filtration through a dry filter paper completed the preparation of the extract for sugar analysis.

Reducing Sugars. The reducing sugars were determined by the very excellent method recently devised by Shaffer and Hartmann (42), in which the cuprous oxide is determined directly by iodo-thiosulphate titration in the presence of an excess of potassium oxalate. The oxalate inhibits the reaction of cupric ions with soluble iodides. The reduction of Fehling's solution is carried out under the standard conditions prescribed for Munson and Walker's method (Official Methods, A.O.A.C.) and the sugar corresponding to the quantity of cuprous oxide read from the tables. By connecting the gas burners with water manometers, and carefully calibrating them in conjunction with the flasks used for the reductions, it was found possible to keep within 10 seconds of the four minutes prescribed for bringing the solution to boiling.

The thiosulphate was standardised against pure copper by an adaptation of the simple method proposed by Peters (36). Triplicate standardisations by this method varied less than 0.01 mg. copper per c.c. N/10 thio-sulphate.

The accuracy of this sugar method was tested with pure dextrose obtained from the Bureau of Standards. Duplicate portions of 100 mg. and of 150 mg. of the anhydrous sugar were weighed out, dissolved in water and carried through the analytical procedure, with the following results:

Dextrose present	Dextrose found
100	99.8
100	99.6
150	149.4
150	149.2

The majority of the sugar determinations made fell within the limits of these quantities. Aliquots of 10 c.c. of the cleared extract were used for the reductions.

Sucrose. The sucrose was determined by the increase in reduction on inversion of the cleared extract. For inversion the citric acid method of Davis and Daish (8) was employed. Comparative tests with invertase

10 *Winter Wheat Varieties and Winter-Killing*

and with the official hydrochloric acid method gave results varying by less than 0.5 per cent. of the quantity found. It appears, therefore, that sucrose was the only disaccharide present in the leaves.

Starch. A qualitative test for starch, with the usual iodine-potassium iodide solution, was made on the dried, ground residue from the alcohol extraction.

Table I. *Changes in amino nitrogen, total soluble nitrogen and sugars of winter wheat leaves during 5 days' storage in ice chest.*

	Fresh leaves percentage green weight	Stored leaves percentage green weight	Percentage change
Amino nitrogen	0.050	0.067	34.0
Water-soluble nitrogen ...	0.157	0.237	51.0
Reducing sugar as dextrose	3.286	4.174	27.0
Invert sugar as sucrose ...	4.574	2.496	52.0
Total sugar as dextrose ...	7.913	6.402	19.4

Table II. *Physical constants of sap of winter wheat leaves.*

Collected October 29, 1920*.

Variety	Classification	Δ	P	pH
Turkey ...	Tender	2.08	24.99	6.360
Kanred ...	Tender	1.97	23.68	6.632
Minhardi (<i>a</i>)	Very hardy	2.41	25.35	6.528
Minhardi (<i>b</i>)	Very hardy	2.45	25.83	6.580
Bufum ...	Very hardy	2.24	26.55	6.465
Odessa ...	Very hardy	2.09	25.44	6.457
Padua ...	Very hardy	2.05	24.63	6.475
Minturki ...	Hardy	2.07	24.87	6.536
Red Rock ...	Tender	2.02	24.28	6.632

* Δ determined directly in tissue by thermoelectric method; pH determined on sap expressed after grinding tissue without previous freezing.

Table III. *Physical constants of sap of winter wheat leaves.*

Variety	Δ	P	$K \cdot 10^4$	pH
Collection of November 12, 1920*.				
Turkey ...	2.26	27.15	11.86	5.840
Kanred ...	2.35	28.23	14.31	6.204
Minhardi (<i>a</i>)	2.56	30.74	13.25	6.078
Minhardi (<i>b</i>)	2.44	29.30	11.87	6.086
Bufum ...	2.68	32.17	14.83	5.980
Collection of December 9, 1920†.				
Turkey ...	1.99	23.92	14.94	5.486
Kanred ...	1.42	17.08	14.04	5.557
Minhardi (<i>a</i>)	1.83	22.00	13.93	5.548
Minhardi (<i>b</i>)	1.69	20.32	15.02	5.733
Bufum ...	1.60	19.24	13.35	5.593

* Sap expressed under pressure of 400 atmospheres after freezing tissues.

† Sap expressed by large hand screw press after boiling tissues in pressure flasks.

Table IV. *The role of sugars and electrolytes in osmotic pressure.*

Variety	P	P_s	$P - P_s$	$K \times 10^3$	K_0	10^3	$\frac{P - P_s}{K \times 10^3}$	$\frac{P - P_s}{K_0 \times 10^3}$
Collection of November 12, 1920.								
Turkey ...	27.15	11.50	15.65	11.86	16.15	1.32	0.97	
Kanred ...	28.23	9.51	18.72	14.31	18.07	1.31	1.01	
Minhardi (a) ...	30.74	11.54	19.20	13.25	17.89	1.45	1.07	
Minhardi (b) ...	29.30	10.51	18.79	14.87	19.34	1.26	0.97	
Buffum ...	32.17	12.32	19.85	14.83	20.71	1.34	0.96	
Average ...	29.52	11.08	18.44	13.82	18.43	1.34	1.00	
Collection of December 9, 1920.								
Turkey ...	23.92	7.49	16.43	11.94	17.98	1.10	0.91	
Kanred ...	17.08	5.40	11.68	14.04	15.97	0.83	0.73	
Minhardi (a) ...	22.00	7.91	14.09	13.93	16.86	1.01	0.84	
Minhardi (b) ...	20.32	6.59	13.73	15.02	17.69	0.91	0.78	
Buffum ...	19.24	7.27	11.97	13.35	16.04	0.90	0.75	
Average ...	20.51	6.93	13.58	14.26	16.91	0.95	0.80	

Table V. *Nitrogen of winter wheat leaves.*

Variety	Dry matter content %	Amino nitrogen		Water-soluble nitrogen		Total nitrogen	
		Green %	Dry %	Green %	Dry %	Green %	Dry %

Collection of November 12, 1920.							
Turkey ...	37.54	0.054	0.14	0.126	0.34	1.394	3.71
Kanred ...	32.89	0.045	0.14	0.129	0.39	1.242	3.78
Minhardi (a)	37.99	0.051	0.13	0.161	0.42	1.416	3.73
Minhardi (b)	33.51	0.048	0.14	0.153	0.46	1.228	3.66
Buffum ...	35.83	0.046	0.13	0.141	0.39	1.181	3.30
Collection of December 9, 1920.							
Turkey ...	29.65	0.049	0.17	0.159	0.54	1.179	3.98
Kanred ...	25.48	0.050	0.20	0.134	0.53	0.936	3.67
Minhardi (a)	31.74	0.058	0.18	0.192	0.60	1.276	4.02
Minhardi (b)	28.20	0.065	0.23	0.175	0.62	1.049	3.72
Buffum ...	29.17	0.058	0.20	0.149	0.51	0.938	3.22

Table VI. *Sugar content of winter wheat leaves.*

Variety	Dry matter content %	Reducing sugar as dextrose		Invert sugar as sucrose		Total sugar as dextrose	
		Green	Dry	Green	Dry	Green	Dry
		%	%	%	%	%	%
Collection of November 12, 1920.							
Turkey ...	37.54	2.992	7.97	5.301	14.13	8.397	22.37
Kanred ...	32.89	3.090	9.39	3.900	11.86	7.055	21.45
Minhardi (<i>a</i>)	37.99	3.237	8.52	4.797	12.63	8.127	21.39
Minhardi (<i>b</i>)	33.51	3.335	9.95	4.351	12.98	7.760	23.16
Buffum ...	35.83	3.308	9.23	5.799	16.19	9.219	25.73
Collection of December 9, 1920.							
Turkey ...	29.65	2.347	7.92	3.598	12.14	6.004	20.25
Kanred ...	25.48	1.840	7.22	2.660	10.44	4.540	17.82
Minhardi (<i>a</i>)	31.74	2.579	8.13	3.356	10.57	5.991	18.88
Minhardi (<i>b</i>)	28.20	2.027	7.19	3.391	12.03	5.472	19.40
Buffum ...	29.17	2.163	7.42	3.772	12.93	5.997	20.56

DISCUSSION.

The depression of the freezing point Δ , the corresponding osmotic pressure P , and the hydrogen ion concentration of the sap of the samples collected October 29 are given in Table II. The osmotic pressures recorded are based on the freezing point data, use being made of the published tables of Harris and Gortner (8). The hydrogen-ion concentration is expressed in terms of pH value as read from the tables of Schmidt and Hoagland (11). A classification of varieties, as determined by survival under field conditions during a number of years, was supplied by the Department of Plant Breeding and is included in the table. It will be seen that in this collection at least there are no significant variations in the constants reported which could be correlated with the relative hardiness of varieties.

The same absence of correlation in physical constants, including specific conductivity K , holds true for the collection of November 12, reported in Table III. It may be noted, however, that the concentration of the sap had increased somewhat in the varieties used. In the collection of December 9, included in the same table, all varieties exhibit a falling off in the depression of the freezing point and corresponding osmotic pressure, probably due in part to simple dilution of the sap, as the moisture content of the tissue was greater. One variety, Kaured, fell off in this respect appreciably more than the rest. It is perhaps noteworthy that this variety winter-killed somewhat more than the others during the year of this test.

An unexpected difficulty was encountered in expressing the sap from the samples collected December 9. The samples were frozen by the method already described, using a freezing mixture of pulverised calcium chloride and snow mixed in the proportions which should give theoretically a cryohydrate mixture with a corresponding temperature of -51.9°C . After seven hours' freezing, the samples were thawed under running water, and refrozen for a period of 11 hours. They were thawed again under the tap. Even after this treatment it was found impossible to express more than 2 to 3 c.c. of juice from 100 grams of material under 100 atmospheres pressure. Further, the plants retained their bright green colour instead of assuming the watersoaked appearance characteristic of frost-killed tissues. The data of Table V show that these samples contained a lower percentage of dry matter than did the earlier collection, consequently the failure to express the sap was not due to lack of moisture in the tissues but apparently to a failure to break down the colloidal

complex of the protoplasm and to increase the permeability of the cell by freezing. Since the permeability of the tissues had not been affected by freezing, it was decided to attempt to destroy the colloidal complex and increase permeability by placing the material in a closed pressure flask and heating it in a boiling water bath. The juice was then expressed as readily by the hand press as by the hydraulic press, 30 to 40 c.c. (about the usual amount) being collected from each sample.

These observations give rise to some very important considerations. In the first place, the wheat plants were apparently not killed by exposure to temperatures lower than normally obtain in many places where they suffer severe winter-killing. The specific temperature must be only one of a number of factors involved. It is also apparent that the hardening process continued long after the advent of freezing weather, as this difficulty was not met with in the collection of November 12. Further, and contrary to the findings of a number of workers, hardening was not in this case associated with an increase in the dry matter content, since as already noted the water content was greater in the collection of December 9. Nor was it associated with an increase in sugar content: this value had decreased, as will be seen later.

Most significant is the evident tenacity with which the hardened tissue grips its water content. Wiegand (18) noted that as the temperature falls the quantity of water separating in the form of ice becomes constantly less and less. The same author develops the theory that the passage of water from the cell during freezing is due to an equalising of the force of imbibition, acting from the outer cell membrane to the centre of the system; this follows as a consequence of the disturbance of equilibrium set up by the force with which the formation of ice crystals takes water from the surface of the cell. Wiegand supports the view that death is due to drying of the protoplasm beyond its critical water content. Evidence of other workers as to the importance of resistance to desiccation has been presented in an earlier section of this paper. Whatever may be the precise mechanism by which withdrawal of water brings about disorganisation of the protoplasm, it seems clear at least that hardness must be intimately connected with forces which oppose this desiccation. In the light of our present knowledge of the properties of colloids, it seems most probable that the principal force is imbibition.

Spoehr (45) found that in cacti the pentosans increase with decreasing water supply. MacDougal (27) also points out that the conversion of the diffusible sugars to the mucilaginous pentosans is one of the alterations which may result in the cell as a consequence of partial desiccation. The

latter author pictures plant protoplasm as a pentosan-protein colloid, and considers that the character and amount of the pentosans largely determine the hydration reactions of the protoplast. Winter conditions where the soil freezes solid are really xerophytic conditions, since the plant's usual water supply is cut off. Having this in mind, it may be reasoned from the evidence of MacDougal and Spoehr that under winter conditions pentosans would accumulate in the cell, contributing largely to the formation of a protoplasmic gel of high imbibitional powers. Investigation of this point will be reserved for a later paper.

The relative importance of sugars and electrolytes in osmotic pressure is considered in Table IV. Unfortunately there is no known method whereby the relative proportions of osmotic pressure contributed by electrolytes and non-electrolytes can be calculated accurately from conductivity and freezing point data. These proportions have sometimes been estimated by taking the value for electrolytes as equal to that of a solution of potassium chloride having the same conductivity. But it is manifestly unjustifiable, as Miss Haynes (22) has pointed out, to assume that the cell sap electrolytes are dissociated to the same extent as potassium chloride, and produce ions of the same mobility. In the present instance the sugar percentages were known and these were assumed to represent the non-electrolyte materials. The theoretical osmotic pressure exerted by the sugars is calculated from the quantities of reducing sugars and sucrose determined by analysis to be present. The difference between this value P_s and the total osmotic pressure P (i.e. $P - P_s$) may be attributed chiefly, though probably not entirely, to electrolytes. In the collection of November 12, the sugars present were sufficient to account for an average of about 38 per cent. of the osmotic pressure; in that of December 9, for about 31 per cent.

The probable effect of non-electrolytes in depressing the conductivity cannot be determined by ordinary viscosity measurements, as these do not distinguish between substances in molecular solution and colloids, and the latter substances, as is well known, do not interfere appreciably with conductivity¹. However, the correction suggested by Miss Haynes for the depression of the conductivity by the sugar content of the sap has been applied to the observed values. The corrected specific conductivity K_0 is derived from the observed value K , by the formula

$$K_0 = \frac{100K}{100 - ax},$$

where $a = 2$ (constant for sugar) and x = per cent. sugar in sap.

¹ For example, see Mason (28), Table V.

The ratio of the value $P - P_s$ to the corrected value for specific conductivity ($\times 10^3$) is given in the last column of the table. This should be a constant in case the cell sap electrolytes in each variety were identical in composition, and the effect of solutes other than sugars on the mobility of the ions can be considered equal for all varieties. The ratio of $P - P_s$ to the observed value for conductivity is given in the adjoining column, in order that the effect of the correction for sugar content may be seen. For the earlier collection the ratio, based on the corrected values for conductivity, varies from 0.96 to 1.07, and averages 1.00, but for the later collection it falls off somewhat, ranging from 0.73 to 0.91 with an average of 0.80. Possibly the heating of these later samples to 100° C. had released ions which would otherwise have remained adsorbed by cell colloids, thus increasing somewhat the value of K , though Mason's results (28, Table VI) suggest that such an increase would probably have been very small. In any case, the diminution in the value of $P - P_s$ in the later collection is so decided as to make it unlikely that the possible disturbing factors introduced by the heating could account entirely for the failure of K to diminish correspondingly. Furthermore, the content of soluble protein (see Table V) is small and relatively constant, and the hydrogen-ion concentration is quite constant, so that the possibility of any considerable variation in conductivity due to protein salts and organic acids may be excluded. The evidence suggests that substances other than electrolytes, and variable in nature, must contribute somewhat to the quantity $P - P_s$, or in other words that sugars are probably not the only non-electrolytes which contribute to the osmotic values.

The amino nitrogen, water-soluble nitrogen and total nitrogen in percentage of green and dry weights are given in Table V. The percentage of dry matter content is also included. It cannot be said that any of the figures exhibit a marked correspondence to differences in degree of hardness. The increase in the water content of the collection of December 9 is accounted for by a mild rainy period of some days' duration which occurred during the previous week. It has been remarked already that Kanred killed somewhat more than the other varieties during the season of this experiment, and this variety was lowest in dry matter content. However, in the light of the evidence already presented, it seems possible that our view of the nature of the correlation between dry matter content and hardness may require modification. A smaller water content is naturally associated with the smaller cells and denser tissues characteristic of the slower growth in late autumn, so that in general hardened tissues would be expected to contain less moisture than

unhardened tissues. But in comparing hardened tissues of different varieties, assuming them to be of similar structure, the moisture content is perhaps largely a function of the relative imbibitional powers of the cell colloids and the degree of previous exposure to modifying environmental factors, for example, desiccating agents such as winds and low temperatures. It seems, therefore, that both the magnitude and order of the values in any particular series of samples may be affected by the weather conditions preceding the date of collection.

The fundamental importance of resistance to withdrawal of water does not, of course, exclude the possibility of an important relationship between the character or state of the proteins and the secondary effects following desiccation. In this instance, though the percentages do not correspond uniformly to the known hardness, yet Minhardi, the hardest variety used, had somewhat the largest content of water-soluble nitrogen. All varieties show an increase in amino nitrogen and water-soluble nitrogen in the later collection. This is in harmony with the evidence of Harvey (19) that splitting of the proteins is associated with the hardening process.

The high content of sugars, especially sucrose, reported in Table VI, is quite remarkable. It has been noted already that sucrose was apparently the only disaccharide present. But here again the varieties could not be classified according to hardness on the basis of the values found. All suffered a loss between the collections of November 12 and December 9, the percentage falling lowest in Kanred. The greater degree of killing in this variety thus lends support to the observation of Pantanelli (25) that hardness was proportional to the quantity of sugar retained during exposure to low temperatures.

A qualitative test for starch on the dried, ground residue from the alcohol extraction gave entirely negative results in every case. This is as expected from the observations of Miyake (32), Lidforss (26) and others.

The above discussion indicates that we are still far from an exact analysis of the factors influencing winter hardness, but certain of the observations, notably the failure of freezing the tissues to break down the protoplasmic structure in the hardened plants, are very suggestive. Further investigation of this phenomenon will be carried out in the near future.

SUMMARY.

1. A number of varieties of winter wheat, known to vary considerably in degree of winter hardiness, were compared in the hardened condition with reference to the physical constants of the cell sap, and the content of dry matter, nitrogen, sugars and starch.

2. No constant relation was found between depression of the freezing point, specific conductivity, or hydrogen-ion concentration of the cell sap and relative frost hardiness.

3. Sugars accounted for 34 to 38 per cent. of the total osmotic pressure of the sap.

4. The ratio of that part of the osmotic pressure not due to sugars (i.e. $P - P_s$) to the corrected specific conductivity ($\times 10^3$) is not a constant. For the samples collected November 12, this ratio varied from 0.96 to 1.07 (average 1.00) and for those collected December 9, from 0.73 to 0.91 (average 0.80).

5. The relation between dry matter content and hardiness was not constant, though one of the two tender varieties had the lowest percentage.

6. All varieties increased in amino nitrogen and water-soluble nitrogen during the hardening process. The hardest variety had the largest content of water-soluble nitrogen, but the relation was not uniform throughout the series.

7. The sugar content did not correspond uniformly with the known hardiness. The percentage decreased between November 12 and December 9, falling lowest in one of the two tender varieties.

8. Sucrose is an important storage material and is apparently the only disaccharide present.

9. All varieties were entirely free from starch.

10. The colloidal complex of the cell of the fully hardened tissue could not be broken down by exposure to the temperature of a calcium chloride-snow cryohydric mixture (theor. = $-54.9^\circ \text{C}.$).

11. The hardened tissue retains its water content with great force. From tissue containing about 70 per cent. of moisture no appreciable amount of sap could be expressed by 400 atmospheres' pressure, even after severe preliminary freezing.

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ON THE SUSCEPTIBILITY OF CLOVER AND SOME
OTHER LEGUMES TO STEM-DISEASE CAUSED BY
THE EELWORM, *TYLENCHUS DIPSACI*, SYN.
DEFLASTATRIX, KÜHN.

By T. GOODEY, D.Sc.

*Department of Helminthology, London School of Tropical Medicine,
late of the Rothamsted Experimental Station.*

(With Plate I, and Tables I and II in Text.)

CONTENTS.	
	PAGE
Introduction	20
The parasite	21
Description of the disease	22
Experimental	23
Discussion	28
References	30
Explanation of plate	30

INTRODUCTION.

It has been known for a number of years that red clover and certain other cultivated leguminous plants, besides many other non-leguminous ones, are subject to attack from the eelworm, *Tylenchus dipsaci*.

Kühn (1881) gave an account of the disease produced in clover and lucerne. Later on Ritzema Bos (1892) showed that the worm attacking clover was morphologically indistinguishable from that attacking rye, oats, hyacinth, carnation and several other cultivated plants and certain weeds. In England Miss Ormerod (1886-1900) dealt with the subject in many of her annual reports and gave a good account of the symptoms produced by *Tylenchus dipsaci*, pointing out in 1899 the differences between these symptoms and those produced by the fungus *Sclerotinia trifoliorum*.

More recently Amos (1919) has published a paper dealing with the difficulties of growing clover, in which he states in general terms the comparative susceptibility of a number of different kinds of clover and other leguminous plants. Also Byars (1920) and Smith (1919) have quite

recently given accounts of the serious damage to red clover in certain areas of the north-west States in the United States of America due to *Tylenchus dipsaci*.

A detailed investigation of the parasitism of *Tylenchus dipsaci* seemed highly desirable, and as a beginning the question of the susceptibility of a number of clovers and other legumes to attack from the worm was taken up.

In the following pages, which are to be looked upon as a preliminary communication only, an account is given of the attempt which I have made to obtain a numerical expression of susceptibility and arrive at some figure which may be considered as an *index of susceptibility* for a given kind of clover or other host plant.

The investigation was undertaken in 1920 when I was acting as helminthologist on the staff of the Institute of Plant Pathology at the Rothamsted Experimental Station, Harpenden, whilst some of the material has been worked up since I was transferred to this laboratory.

I wish to acknowledge my indebtedness to Mr R. A. Fisher of the Rothamsted Laboratory for kindly working out the Standard Error in connection with the percentages of deformed seedlings, and for some suggestions. My best thanks are also due to Mr Arthur Amos who helped me by supplying diseased clover plants and to Messrs Sutton & Sons who very kindly sent the seeds used in these experiments.

THE PARASITE.

The parasite causing the disease is a small nematode worm belonging to the family *Anguillulidae*, popularly known as eelworms. It is not my intention in the present paper to give a detailed description of the worm; this has been done by many previous investigators whose works may be consulted; good accounts of it are to be found in Ritzema-Bos (1892) and Marcinowski (1909).

The adults are visible to the naked eye when seen suitably illuminated, and measure from one millimetre to one and three-quarter millimetres in length. The widest part of the body is about one-fortieth of the total length.

The sexes are distinct and the males are on the whole a little shorter and narrower than the females. Although, as stated above, the worm has been described many times previously, there is still need for much information concerning the structure of several of its organs. Moreover, we need to know exactly how the parasite gains access into the tissues of the host plant, and also how it uses the chitinous stylet which it carries

in the buccal cavity. It is usually assumed that it is by means of this organ that it pierces plant tissues and then penetrates into the plant, and that having once got inside it uses its stylet to puncture the cells amongst which it is lying, and feeds on the cell sap which it sucks out from them.

All this, however, needs much further investigation, I think, especially when one remembers the power possessed by the infective larvae of the human parasite *Ancylostoma duodenale* of boring through the skin without the aid of any piercing mouth armature. Moreover, no one, as far as I know, has ever observed the stylet of *Tylenchus dipsaci* in use as a piercing organ. It is a very small structure, being 12-15 microns in length, and the point is so fine that it can only be seen under the very high magnification of an oil-immersion lens. In all my numerous examinations of diseased clover plants, both fresh and preserved, I have never found the stylet exerted from the anterior end of the body.

Another point: it is a matter of observation that the parasite, in some way or other as yet not understood, causes an increase in the size of parenchymatous cells of the host plant, and this also requires further investigation. Ritzema-Bos suggested that it was due to a secretion poured out by the worm, and considered the spatulate posterior portion of the oesophagus as probably the seat of the secreting gland: the matter is, however, still very obscure. It is hoped that later on one may be enabled to take up the elucidation of these interesting problems.

DESCRIPTION OF THE DISEASE.

In regard to a suitable name for this disease, the term *Stem disease* should be used, thus keeping in line with the German expression *Stockkrankheit* for the same condition. The term *Stem-rot* is already in use as the name of the fungal disease of clover caused by *Sclerotinia trifoliorum*, see Amos (1919) and Cotton (1920).

Admittedly the two names are sufficiently alike to lead to the possibility of confusion in nomenclature for two very different pathological conditions, but I would suggest that the confusion might be avoided by the use of another name for the fungal disease which is not strictly confined to the stem as is the eelworm disease, but attacks the whole of the foliage of the plant.

The term *Foliage-rot of clover*, or *Clover rot* would be preferable to *Stem-rot*, which is not an exact descriptive designation. It is of interest to note that the Germans have avoided confusion by the use of the word *Kleekehrs* for the fungal disease.

The following is a brief account of the chief characteristics of the two diseases, the differences between which are fairly well shown in the two photographs at the end of this paper.

Stem disease caused by *Tylenchus dipsaci* is chiefly characterised by a stunting and deforming of the plant accompanied by much swelling of the leaf stalks at their base and of the stipules. The older leaves die back and new leaves which develop, though frequently numerous, have very short stalks, and the leaf blades are twisted and much wrinkled. The leaf stalks and stipules are also very much discoloured. The plant gradually dies back and is eventually killed, though the parasite does not attack the root.

The disease spreads slowly from an infective centre and persists throughout the year, being most noticeable perhaps during the winter and spring when the leafage is not profuse.

Foliage rot of clover, or *Clover rot*, due to the fungus, differs from the eelworm disease in the appearance of the affected plants. The foliage is first attacked and becomes covered with a white mould or mycelium which causes the leaves and stems to turn brown and rot rapidly. The spread of the disease is rapid, sweeping over a whole field in a few days, especially when favoured by mild damp weather during autumn and winter. There is no stunting or swelling of the affected parts but just a collapse and decay of the foliage followed by attack on the roots.

If not too badly attacked, a second crop of clover may spring up from the unkilld roots and a fair yield may be obtained.

The disease does not apparently continue to attack the clover during spring and summer.

A certain amount of confusion seems to exist among agriculturists as to the differences between these two diseases, but to anyone who has seen the two conditions in the field the differences are very marked and unmistakable. The confusion is partly explicable on account of the frequent association of saprophytic eelworms along with the fungal foliage-rot disease. All these eelworms are different, however, from the disease producing *Tylenchus dipsaci* and are free-living soil nematodes which have invaded the rotting clover tissue and have there found a highly favourable medium for growth and multiplication.

EXPERIMENTAL.

Some preliminary experiments were carried out in which several seedlings of red clover, about 10 days old and showing the first true leaf, were each inoculated with a single egg containing a well developed larva.

The inoculation was carried out by means of a fine capillary glass pipette. Later examination and dissection of seedlings revealed the fact that mature and sexually differentiated worms were developed in from 24 to 30 days from the date of inoculation.

Several experiments were also made in which clovers of different kinds were grown on soil heavily infected with *Tylenchus dipsaci*. It was invariably found that the seedlings of red clover showed a high percentage of affected plants which possessed, at first, swollen hypocotyls and later on became stunted and deformed with small wrinkled leaf-blades.

Following these early experiments the main experiment which forms the basis of this paper was set up.

The soil used was that from around the roots of diseased red clover from the University Farm at Cambridge, which had been kept for about two months at the bottom of a small galvanised iron bin. All the plants had died down and become withered and brown.

The soil was crumbled up finely and the tops of the plants were chopped up finely and thoroughly mixed into the soil, with which also a small proportion of silver sand was incorporated.

This mixture, which it was considered would be highly infective for *Tylenchus dipsaci*, was put in a layer about one inch deep on the top of Hoosfield soil plus 10 per cent. sand in 10-inch glazed pots, thus forming a shallow layer of infective soil close to the top of the pot.

Each pot was divided into four quadrants for economy of space by means of glass partitions pressed down into the soil.

One hundred seeds of each of the following were sown, each in its quadrant, and covered with a thin layer of sand.

Red clover—English, French, Canadian, Wild English.

Cow-grass—English, Swedish.

Alsike clover—English, Canadian.

White clover—Sutton's Mammoth, Wild Cotswold, Wild Kentish, English, Dutch White.

Kidney vetch, *Sainfoin*, *Lucerne* (Provence), *Trefoil*.

All the seeds were sown on the same day, June 1st. The pots were then carefully watered and put out in the wire-covered enclosure and there left for the seedlings to grow under the same conditions of temperature, light, air, moisture, etc. It was hoped by giving throughout all the pots uniform conditions of soil in which the parasites were as equally distributed as could be arranged, and by leaving them under the same climatic conditions in all cases, that any differences shown in the inci-

dence of attack by *Tylenchus* would be due to differences of susceptibility to the parasite. That this was a reasonable and sound assumption is borne out, I think, by the results obtained.

On July 8th, that is, after 37 days, all the seedlings were harvested, counted, and after being separated into deformed and healthy in appearance, were separately pickled in 70 per cent. alcohol.

The figures obtained in this way enabled me to arrive at the percentage of deformed seedlings in each case, as shown in the following table.

Table I. *Showing the numbers of healthy and deformed seedlings, the percentage of deformed, and the standard error.*

Kind of plant	No. of seedlings			Total	Percentage of deformed	Standard error	
	Healthy	Deformed					
<i>Red clover:</i>							
English	35	41	76	54	5.7
French	26	61	87	70.11	4.9
Wild English	18	47	65	72.3	5.5
Canadian	19	61	80	76.25	4.8
<i>Cow-grass:</i>							
English	33	39	72	54.1	5.9
Swedish	26	37	63	58.7	6.2
<i>Alsike clover:</i>							
English	71	12	83	14.4	3.9
Canadian	60	23	83	27.7	4.9
<i>White clover:</i>							
Sutton's Mammoth	79	9	88	10.2	3.2
English	53	10	63	15.87	4.6
Wild Cotswold	72	10	82	12.2	3.6
Wild Kentish	77	10	87	11.5	3.4
<i>Kidney vetch</i>	31	42	73	66.6	5.8
<i>Sainfoin</i>	58	13	71	18.3	4.6
<i>Lucerne</i> (Provence)	68	4	72	5.2	2.7
<i>Trefoil</i>	41	0	41	0	—

These figures of percentage infection give one a rough indication of the susceptibility of the different kinds of clovers, etc., but it was thought that if one could estimate the numbers of *Tylenchus* in a series of deformed seedlings one would be able to arrive at some expression for the intensity of infection or intensity of susceptibility in each case.

With this end in view I examined all the deformed seedlings and selected the ten most deformed in each case, and then proceeded to the dissection of these in order to obtain the contained *Tylenchus*.

Each seedling was carefully dissected by means of needles, with the aid of a binocular dissecting microscope. This, of course, was a slow

process, but it was found quite practicable, and comparatively few of the worms were damaged or broken during these operations. Each seedling was placed in a shallow glass capsule in distilled water, and bit by bit dissected so as to free the tissues from the contained parasites which were then left in the water whilst the vegetable matter was gradually removed.

After the dissection the nematodes were collected, concentrated and transferred to a microscope slide or slides as the case might require, and then examined under the microscope. For the purposes of collection I first of all used glass capillary pipettes, but later on abandoned these and used the centrifuge which proved very serviceable. The resulting drop for examination thus contained adults of both sexes, larvae in many stages of development, and numerous eggs. In making my examinations I recorded separately the numbers of males, females and larvae; eggs were not counted. A record was also kept of whatever other kinds of nematodes might be present with the *Tylenchus*. These, however, do not concern us in the present paper.

In the case of those varieties which gave a large number of deformed seedlings, the ten most deformed were, as stated above, selected for dissection, whilst in the other cases as for example, lucerne, sainfoin, white clover, where only a few seedlings were deformed, the whole of them were dissected.

In the case of lucerne only four seedlings were deformed, but none of these revealed any adult *Tylenchus* on dissection.

Since the same number of seedlings was not dissected throughout, it became necessary to decide arbitrarily on some number to take as an average in arriving at the index of susceptibility, and for this purpose the number of deformed lucerne seedlings was chosen, i.e. four.

Since also index of susceptibility was being interpreted as equivalent to intensity of susceptibility, the four highest totals of males plus females were taken and an average made of these, the resultant figure being called the index of susceptibility. Whether the males and females counted are the progeny of a single fertilised female, thus giving a reproduction figure, or whether they are the result of an invasion of the tissues of the host by numerous larvae which have attained sexual differentiation and maturity within the seedling, the data available are not sufficient for one to say, since we do not know enough of the life-history of the parasite to say definitely which is the infective larval stage.

The figure arrived at in each case gives us, I think it will be agreed, an expression of the suitability of the host plant for the needs of the parasite, and this is what the investigation was designed to reveal.

Table II. Showing the four highest counts of male and female *Tylenchi* in seedlings, the average of the totals of these giving the index of susceptibility in each case.

Name of plant				Males	Females	Total		
<i>Red clover</i> , English	103	136	239		
				90	104	194		
				66	100	166		
				68	93	161		
Average						190	index of susceptibility	
<i>Red clover</i> , French	110	250	360		
				63	96	159		
				44	108	152		
				9	140	149		
Average ...						205
<i>Red clover</i> , Wild English	35	112	147		
				39	136	175		
				30	112	172		
				30	116	146		
Average ...						160
<i>Red clover</i> , Canadian	87	317	404		
				72	180	252		
				68	264	332		
				96	180	276		
Average ...						316
<i>Cow-grass</i> , English	33	84	117		
				4	11	15		
				4	6	10		
				2	9	11		
Average ...						38.25
<i>Cow-grass</i> , Swedish	49	80	129		
				55	98	153		
				59	67	126		
				100	160	260		
Average ...						167
<i>Alsike clover</i> , English	10	10	20		
				10	25	35		
				13	12	25		
				20	14	34		
Average ...						28.5
<i>Alsike clover</i> , Canadian	18	30	48		
				16	46	62		
				7	17	24		
				5	9	14		
Average ...						37
<i>White clover</i> , Sutton's Mammoth				nil	nil	nil		
<i>White clover</i> , English	6	4	10		
				1	5	6		
				2	—	2		
				—	1	1		
Average ...						4.75

Table II (*continued*).

Name of plant				Male	Females	Total		
<i>White clover</i> , Wild Gotswood	5	7	12		
				1	7	8		
				1	1	2		
Average			5.5	index of susceptibility	
<i>White clover</i> , Wild Kentish		1	1		
				2	3	5		
				1	—	1		
				—	1	1		
Average			2
<i>Kidney vetch</i>	41	67	108		
				44	78	122		
				85	127	212		
				95	118	213		
Average			163.75
<i>Sainfoin</i>	1	6	10		
				4	2	6		
				7	3	10		
				—	2	2		
Average			7
<i>Lucerna</i>	nil	nil	nil		
<i>Trefoil</i>	nil	nil	nil		

DISCUSSION.

In a biological investigation of this character it is practically impossible to repeat the experiments exactly, and consequently one would not expect to get the same figures in a repeat experiment.

Nevertheless, one would, I think, obtain figures having the same relative significance, and for this reason the figures put forward above as indices of susceptibility may be taken as representing approximately the relative susceptibilities of the different clovers, etc. to *Ty. dipsaci* attack.

An examination of the figures shows that all the varieties of red clover tested are very susceptible to attack and fall into a common group (1) to which also belong cow-grass (Swedish) and kidney vetch.

Arranged in order of intensity of susceptibility we have them as follows:

Red clover (Canadian)	...	316	} Group 1.
.. (French)	...	205	
.. (English)	...	190	
Cow-grass (Swedish)	...	167	
Kidney vetch	...	163.75	
Red clover (Wild English)	...	160	

Widely separated from this group we have another one (2) much less susceptible, comprising

Cow-grass (English) ...	38.25	} Group 2.
Alsike clover (Canadian)...	37	
Alsike clover (English) ...	28.5	

The great disparity between the figures for the Swedish and the English cow-grass is remarkable and somewhat surprising, and suggests the need for much further investigation along these lines.

A further group (3) comprises varieties which are but very slightly susceptible to attack, viz.:

Sainfoin	7	} Group 3.
White clover (Wild Cotswold)	5.5	
.. (English) ...	4.75	
.. (Wild Kentish) ...	2	

Lastly we have a group (4) made up of

White clover (Sutton's Mammoth)	} Group 4.
Lucerne	
Trefoil	

which appear to be insusceptible to attack.

A comparison of the indices of susceptibility with the percentages of deformed seedlings shown in Table I reveals the fact that all the members of Group 1 have a high percentage of deformed seedlings. Canadian red clover, having the highest index of susceptibility, has also the highest percentage of deformed seedlings. The parallel does not hold throughout the group, however, for Wild English red clover, which has the second highest percentage of deformed seedlings, has the lowest index of susceptibility in Group 1.

The low index of susceptibility shown by English cow-grass, compared with its high percentage of deformed seedlings, is an outstanding exception to the parallelism of high index of susceptibility with high percentage of deformed seedlings.

In the case of Groups 2 and 3 it is not possible to establish a parallel when we have cases like English alsike clover with a lower percentage of deformed seedlings than either English white clover or sainfoin, and a much higher index of susceptibility than either of these.

These results are in general agreement with those of Amos (1919, pp. 8 and 9), except as to one or two details. He never found sainfoin attacked, whilst I find that the seedlings are very slightly susceptible to attack.

The results have a practical bearing of considerable importance to the farmer whose land is infested with *Tylenchus dipsaci*, and whose red clover is therefore liable to attack from this parasite.

If he wishes to avoid Stem disease he should not sow red clover, cow-grass or alsike clover, but should make use of trefoil, lucerne, sainfoin or a large white clover, such as Sutton's Mammoth White.

Recommendations similar to the above have been made before, notably by Amos (1919, p. 9), but they are so obviously the proper and hopeful lines to adopt that they will bear repetition and reiteration. It is no use attempting to get rid of the eelworm disease if red clover, cow-grass, alsike clover and kidney vetch are sown on infected land.

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EXPLANATION OF PLATE I.

FIG. 1. Photograph of portions of plant of red clover suffering from Stem disease caused by *Tylenchus dipsaci*, showing the characteristically swollen leaf stalks and stipules at A and B.

FIG. 2. Photograph of a whole plant of red clover suffering from Foliage rot caused by the fungus *Sclerotinia trifoliorum*. The white mycelium can be seen spreading on to the surface of the soil, whilst the collapsed nature of the leaf and leaf-stalks is well shown.

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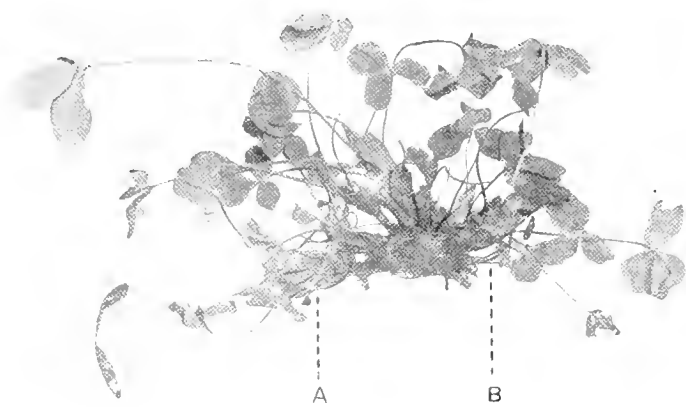


Fig. 1.



Fig. 2.

GENETIC STUDIES IN POTATOES; STERILITY.

BY R. N. SALAMAN, M.A., M.D., *Barley, Herts.*

AND

J. W. LESLEY, M.A., *Plant Breeding Institute, Cambridge University.*

(With Plate II.)

MALE sterility in potatoes, that is the absence of pollen from the anthers, has been shown by one of us (Salaman(1)) to behave as a recessive to male fertility. The present paper is an account of some further experiments in the genetics of this character, particularly of crosses between two varieties in which the reciprocals give different results.

Both quantity and quality of pollen were estimated by methods similar to those used by Salaman(1). Four empirical grades of quantity were employed, viz. "abundant," "medium," "small" and "very few grains." The quality of the pollen was determined by mounting a sample in water and examining microscopically. Under this treatment living and presumably healthy grains swell up, appear spherical and translucent, whilst the pores become prominent. Such grains are termed "good." Bad grains are generally smaller, irregular in outline, and often appear quite empty.

Even the best samples of pollens which we have seen contain a considerable proportion of empty grains. The proportion of good grains was determined approximately by counting.

THE RELATION BETWEEN QUALITY AND QUANTITY OF POLLEN.

A close correlation was found to exist between quantity and quality of pollen as indeed was observed by East(2) and Salaman(1). In general the larger the quantity the higher the proportion of good grains. The *constant* presence of "abundant" pollen is a sure sign of a large proportion of good grains. Similarly the lowest grade of quantity—"very few grains"—is proof of the absence of good pollen. Of the intermediate grades "medium" quantity was generally associated with poor quality pollen. The grade which we describe as "small" rarely contained any good grains. Perhaps "medium" quantity is best counted as potentially fertile, the "small" quantity as potentially sterile.

In some families the difficulty of classification was considerable, and a satisfactory method of dealing with a quantitative and variable character such as the present has yet to be discovered. In some few cases only the quantity of pollen was recorded but as a rule both quantity and quality were estimated on three or four different dates; especially was this so when flowering was continued over a long period (this varied in different seedlings from one to over fourteen weeks).

Frequently the repeated observations both of quantity and quality of pollen agreed closely in different flowers on the same plant but in some cases this was not so. Little is known of the factors causing such variations. The variation of quantity is chiefly to be observed in sterile or partially fertile plants, and may be, in part, a result of the irregular and delayed ripening of the anthers characteristic of such plants. We find the quality of pollen sometimes deteriorates considerably after the flower has been open for five or six days, but apart from that there are inherent differences in the degree of fertility. For instance, *Edgecote Purple* invariably shows abundant pollen of high quality and may be termed fully fertile. *Edzell Blue* on the other hand is somewhat variable in quantity, usually only "medium" and the quality is correspondingly inferior.

We have not attempted to measure small quantitative differences but only large differences of a more obvious kind. The methods of estimation are, of course, approximate and not suitable for accurate measurement, but are sufficiently effective in determining large differences. Rapidity of execution is also a consideration when over three hundred microscopic examinations need to be made in the flowering period.

MATERIAL USED.

Three cultivated varieties formed the basis of the breeding experiments. They are characterised as follows:

(1) *Edgecote Purple*. The male organs are fully fertile, having abundant pollen of good quality of which at least 25 per cent. consists of perfect grains. It forms berries and seed freely by natural self-pollination. Our stock of this was brought by Professor Biffen from Wiltshire, where it maintains a fairly high yield although grown year after year without the usual renewal by means of Scotch or other seed.

(2) *Myatt's Ashleaf*. The male organs are fully fertile like the preceding. It likewise forms berries and seed, self-setting freely.

(3) *Edzell Blue*. The male organs are fertile but not fully so, being considerably less fertile than either of the two preceding varieties. Both

quantity and quality vary considerably, but as a rule it is “medium,” containing about 5 per cent. good grains. Forms berries but sparingly, such berries having fewer seeds than the preceding.

Parchment bags and the usual precautions were used unless expressly noted to the contrary. The seed from each berry was kept separate, which served as a useful check when self-set or “natural” berries were used.

More than 200 selfed seedlings were raised from Edgecote Purple and many of these carried on to the second year by tubers. Twenty-five plants flowered which were recorded as follows:

Abundant	...	20	Small	1
Medium	...	3	Very few grains			1

All of the nine examined qualitatively contained good pollen (see Table I). Two plants, one with “small” quantity, the other with “very few grains,” were only once recorded and are of doubtful significance. Fertility, which has already been shown to be recessive in the potato, here probably breeds true in accordance with expectation. Five individuals formed self-set berries.

Table I.

	Abundant	Medium	Small	Very few grains	Per cent. good.										Small
					Abundant					Medium					
					0	1-	6-	11-	16-	0	1-	6-	11-	16-	
Edgecote Purple selfed ...	20	3	1	1	.	2	1	.	3	.	.	2	.	1	.
Edzell Blue selfed ...	8	6	3	4	.	.	2	1	1	1	2	1	.	.	.
Edgecote Purple × Myatt's Ashleaf ...	19	3	5	3	.
Myatt's Ashleaf × Edge- cote Purple ...	13	1	2
Edzell Blue × Myatt's Ash- leaf ...	8	4	3	10	1	1	1
Edgecote Purple × Edzell Blue ...	44	2	2	2	34
F_2 from Edgecote Purple × Edzell Blue ...	15	1	1	7
Edzell Blue × Edgecote Purple ...	13	10	8	18	2	5	.	.	3	4	6

Some 200 selfed seedlings from Myatt's Ashleaf were raised, mostly in 1921 which was a disastrous year for potato seedlings in this district. Of these only two flowered, both having “abundant” pollen of which some at least was certainly good. The evidence here is very meagre and merely shows that Myatt's Ashleaf gives fertile offspring when selfed.

In the case of Edzell Blue only self-set or “natural” seed has been available notwithstanding that about 100 flowers were self fertilised

under bags. The seed from the natural berries was sown separately giving 150 seedlings; but eight plants from one berry proved from other evidence to be the result of a cross and were therefore not included, otherwise the results are consistent and trustworthy. Twenty-one plants flowered and were classified as follows:

Abundant	...	8	Small	3
Medium	...	6	Nil	1

The qualitative results are shown in Table 1. It was noted that even the fertile plants, unlike those from Edgecote Purple selfed, were apt to vary considerably in different flowers and on different dates both in quantity and quality of pollen. Although itself fertile Edzell Blue selfed actually throws a large proportion of sterile offspring, a result which contrasts strongly with that obtained from Edgecote Purple. A somewhat similar appearance of sterility is recorded by Salaman(1) in certain lines derived from a fertile Sutton's Flourball. We shall submit an explanation after the much fuller evidence from crosses has been given.

Two hundred seedlings were raised from the cross—Edgecote Purple \times Myatt's Ashleaf in 1921. Of the F_1 plants 22 flowered, consisting of

Abundant	...	19	Medium	...	3
----------	-----	----	--------	-----	---

All were fertile and contained at least 20 per cent. of good grains.

A similar number of seedlings were raised from the reciprocal cross—Myatt's Ashleaf \times Edgecote Purple—of which 13 flowered. All were fully fertile. The results from this cross are at once intelligible and harmonise with the few selfed data available, fertility behaving as a recessive.

The cross—Edzell Blue \times Myatt's Ashleaf—was raised in 1920, and grown on in 1921 when a further sowing was also made. Of the 163 plants grown 25 flowered and were recorded as:

Abundant pollen	8	Small	3
Medium pollen	4	Very few grains			10

As in the case of Edzell Blue selfed the quantity varied considerably in different records from the same individual. Unfortunately the quality of pollen here was only examined in three cases (see Table 1). It is clear, however, that a large proportion of sterile plants result from this cross between two fertiles.

In 1919 the crosses—Edzell Blue \times Edgecote Purple and Edgecote Purple \times Edzell Blue—were made. In the families raised in 1920 there

was no significant difference either in the number of seeds per berry or in the germination of the seed or in the mortality among seedlings. Neither flower nor tuber colour showed any significant difference.

Two types of inflorescence occurred in these families. In the "simple" type (see plate) the primary axis divides into several (usually two) secondary axes, each of which forms a scorpioid cyme. In the "compound" type (see plate), however, the two or more secondary axes divide again, forming tertiary axes and some of these divide so that axes of the fourth or fifth order are formed each of the ultimate branches forming a scorpioid cyme as before. Similar types of inflorescence have been studied by Crane(8) in tomatoes. The proportions of these two types in the present case showed no significant difference in the reciprocal crosses.

The proportion of plants which flowered in Edgecote Purple \times Edzell Blue, however, was nearly double that in the reciprocal family. (In 1920, 23 per cent. as against 13 per cent.; in 1921, 72 per cent. as against 37 per cent.) Both families were equally vigorous. In the late summer when many of the plants had ceased to flower several seedlings in Edgecote Purple \times Edzell Blue were seen to bear berries, often a dozen or more on a plant. In the reciprocal cross, on the contrary, extremely few plants had berries and these in but small numbers¹. It occurred to us that this difference might be due to a difference in pollens, as seed and berry production in potatoes is frequently determined by pollen (cf. Stuart(3)). As a first step, all the available pollens were examined. In Edgecote Purple \times Edzell Blue all the 18 plants tested had "abundant" pollen. This was in accord with the frequent presence of self-set berries in this family. In the reciprocal cross 15 plants were recorded where pollen was described as follows:

Abundant	...	1	Small	8
Medium	...	1	Very few grains			5

Only one plant was "abundant" as against the entire 18 in the reciprocal family. A remarkable difference in the male organs of the reciprocal cross thus came to light, a difference which is paralleled by previously observed difference in fruit and seed production. Both families were grown on by tubers in 1921 and a further sowing made of 1919 seed of the cross Edzell Blue \times Edgecote Purple.

¹ The berries both from Edgecote Purple \times Edzell Blue and Edzell Blue \times Edgecote Purple contained seed. The average germination capacity was 90 % and the lowest 38 %. We are indebted to Mr Saunders of the National Institute of Agricultural Botany for testing the seeds.

From the cross Edgecote Purple \times Edzell Blue 44 plants flowered. Both quantity and quality of pollen were repeatedly tested; all were fully fertile. The constant production of "abundant" pollen of high quality was a characteristic of every plant. The results from separate berries were consistent and the observations of 1920 were therefore fully confirmed (see Table 1).

An F_2 family from a fully fertile F_1 plant from this cross was raised in 1921 and 15 plants flowered. All had "abundant" pollen of high quality (see Table 1). Thus there is no evidence here of sterility arising either in F_1 or F_2 .

In Edzell Blue \times Edgecote Purple grown on 19 plants flowered; both quantity and quality were again repeatedly observed with the following results:

Abundant	...	13	Small	8
Medium	...	10	Very few grains			18

In the qualitative test (Table 1) only four of the plants showed any considerable proportion of good grains; the majority of plants tested had no good grains. Again the results from separate berries were consistent. The difference in berry production which first attracted our attention was as remarkable in 1921 as in the previous year. In Edgecote Purple \times Edzell Blue over 50 per cent. of the individuals bore self-set berries, often in the greatest profusion; of those grown under more favourable conditions at Ormskirk, actually 26 out of 28 (over 90 per cent.) bore berries. In the family Edzell Blue \times Edgecote Purple only six plants bore self-set berries and here it is interesting to note the correlation between good quality pollen and the setting of natural berries. All of the four plants having good pollen had self-set berries, but only one of the sixteen plants having no good pollen had self-set berries and even these were probably due to insect-borne pollen. These figures give no idea of the contrast in quantity of berries per plant which was even more remarkable (see Plate). That there is no evidence of female sterility here is indicated by the presence of natural berries on the six plants and on two others which set seed when artificially crossed with fertile plants.

It has therefore been shown that whilst Edgecote Purple \times Edzell Blue gives a wholly fertile F_1 the reciprocal cross gives some sterile and some fertile plants.

DISCUSSION.

The two main facts to which we wish to draw attention are:

(1) The appearance of sterile (or female) plants from the fertile variety, Edzell Blue, when selfed and from crosses between it and other fertiles, Myatt's Ashleaf and Edgecote Purple.

(2) The different results obtained in F_1 from a reciprocal cross.

The evidence certainly points to a particular one of the three varieties as the principal agent introducing sterility. Both Edgecote Purple and Myatt's Ashleaf are themselves remarkably fertile. They have been crossed reciprocally and gave a wholly fertile F_1 ; there is some evidence that when selfed they give fertile offspring. With Edzell Blue it is otherwise. It is itself only moderately fertile. Both selfed and crossed with Myatt's Ashleaf it gives a majority of sterile offspring. It is noteworthy that sterility has arisen in both cases where Edzell Blue is the mother parent. It behaves as a sterile when used as female and as a fertile when used as a male parent.

In plants male sterility arising from a cross between fertile parents is, of course, a well-known occurrence, but in the present case sterility arises from a cross in one direction but not in the other.

This difference in the reciprocal cross between Edzell Blue and Edgecote Purple points to some difference in the eggs and pollen of one or both of the parents. The evidence seems to point strongly to such a difference in the sexual cells of Edzell Blue. It is not impossible that in Edzell Blue the sterility is attached to the cytoplasm of the egg, but that the generative nucleus of the pollen grain carries the basis of fertility. As sterility normally behaves as a dominant we should then expect all the F_1 to be sterile where Edzell Blue is the mother. This, however, was not so. A few were fertile, but we know of no evidence suggesting that the cytoplasm of eggs may differ in constitution. Indeed the heterogeneity of the eggs suggests segregation. It is more likely that a factor or factors are at work which are localised in the nucleus.

As sterility is dominant it would seem that the eggs of Edzell Blue are of two kinds, some—possibly half—carrying male sterility, the remainder carrying male fertility. The data at present available only give an approximate idea of the proportions. The pollen on the other hand all carries fertility. Following the same hypothesis both eggs and pollen of Edgecote Purple and Myatt's Ashleaf would appear to carry fertility.

In order to account for the difference between the eggs and pollen of Edzell Blue we suggest that at some stage of development a process

of segregation has occurred at which the basis of sterility has dropped out of the lineage of the *male* germ cells. On the other hand the germ lineage of the eggs is unaffected so that male sterility is present in some of the egg cells and is absent from others; this may well be merely the result of normal segregation. Possibly in the developing bud at the time when the *male* organs of the flower are being differentiated a differentiation of genetic characters takes place. This hypothesis affords an explanation of the rather paradoxical male fertility of Edzell Blue in spite of its containing the dominant element of male sterility. For as a result of the premature segregation the primordia of the anthers would only contain the basis of fertility and are therefore able to form good pollen. It would account for the fertility of F_1 and F_2 from the cross Edgescote Purple \times Edzell Blue and for the fertility of the reciprocal crosses between Edgescote Purple \times Myatt's Ashleaf. The F_1 result from the cross Edzell Blue \times Myatt's Ashleaf which was similar to that of Edzell Blue \times Edgescote Purple is also intelligible on the above hypothesis. A difficulty is met however in the result from Edzell Blue selfed. According to the theory about half the eggs carry sterility and the other half fertility while all the pollen carries fertility. Consequently selfing should give half sterile and half fertile offspring, *i.e.* the same result as Edzell Blue \times Edgescote Purple. But the data (Table I) indicate an excess of fertiles. The numbers are small but the discrepancy demands further investigation. Again, no explanation is afforded of the rather imperfect pollen production of Edzell Blue.

To sum up it appears that in male sterile varieties the eggs either all carry male sterility or some carry male sterility and the remainder male fertility. The eggs of male fertile varieties (except Edzell Blue) and the pollen of all male fertile varieties tested carry male fertility. As sterility behaves as a dominant the F_1 from a cross, sterile \times fertile, consists of all steriles or partly of sterile and partly of fertile plants.

Miss Saunders(4) in her classical experiments with *Matthiola* has shown that the pollen of certain "singles" is all of one kind, but that the eggs are of two different kinds with respect to the "doubling" factors. Other experiments(5) suggest that in *Petunia* the pollen is heterogeneous and the ovules homogeneous for a "singleness" factor. The present data point to an explanation of the former kind in potatoes.

If we regard a male-sterile plant as a functional female it follows that the gametes of Edzell Blue are differentiated in regard to sex, the eggs carrying either Hermaphroditism (δ Fertility) or Femaleness (δ Sterility) and the sperms all Hermaphroditism (δ Fertility).



Fig. 1



Fig. 2

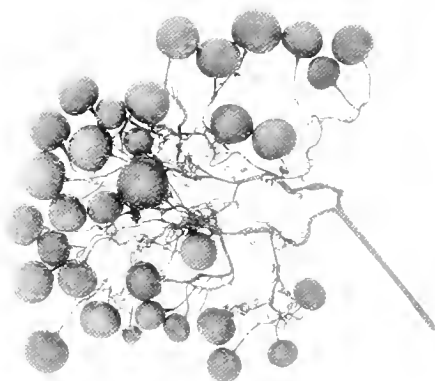


Fig. 3

That a process of differentiation prior to normal segregation may occur in plants is shown by the work of Bateson(6) and (7) and others. In the present case there is reason to suspect a segregation by which sterility is dropped out of the germ lineage of the sperms but not of the eggs.

In concluding it is a pleasure to acknowledge the help and encouragement given us by Prof. R. H. Biffen and the willing assistance of Miss E. Hagger at Barley and Mr Hamilton at Cambridge.

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EXPLANATION OF PLATE II.

- Fig. 1. "Simple" cymose system of male-sterile F_1 plant from Edzell Blue \times Edgecote Purple.
- Fig. 2. "Compound" cymose system of male-sterile F_1 plant from Edzell Blue \times Edgecote Purple showing variation from the less "compound" type at the apex to the more "compound" type at the base of the shoot.
- Fig. 3. Bunch of self-set berries developed from a compound inflorescence of male-fertile F_1 plant from Edgecote Purple \times Edzell Blue.

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A STUDY OF NITROGEN METABOLISM IN THE DAIRY COW.

BY CHARLES CROWTHER, M.A., Ph.D.
AND HERBERT ERNEST WOODMAN, D.Sc., Ph.D.

The work outlined in the present communication was carried out during the years 1916-19 in the Institute for Research in Animal Nutrition of the University of Leeds.

IN an earlier communication¹ dealing with the results of a series of digestibility determinations carried out by us with two sheep, attention was directed to the fact that, when the averages for consumption and excretion of nitrogen in the different periods were arranged in the order of increasing nitrogen consumption, the retention of nitrogen in the body of the sheep rose only up to a certain point and then fell. The essential data are reproduced below:

Period	Nature of ration	Average per day			
		Nitrogen digested		Nitrogen retained (+) or lost (-) by sheep	
		Sheep 1	Sheep 2	Sheep 1	Sheep 2
		gm.	gm.	gm.	gm.
I	Hay + palm kernel cake I ...	6.88	7.38	- 0.37	1.94
IV	Hay alone ...	9.09	9.61	+ 1.93	2.14
II	Hay + palm kernel cakes I and II	11.40	12.48	+ 3.80	4.76
V	Hay + extracted palm k. meal ...	12.22	13.12	+ 3.33	2.15
III	Hay + undec. cotton cake ...	12.31	12.96	+ 3.25	3.76
VI	Hay + dried yeast ...	18.10	18.28	+ 2.94	1.35

It will be noted that each sheep showed the maximum retention of nitrogen in Period II, in which about 12 gm. digestible nitrogen, or roughly 17 gm. total nitrogen (= 106 gm. total crude protein) per head per day (= 2.4 kg. total crude protein per 1000 kg. live-weight) were consumed. In the periods following this in the table, as the nitrogen-consumption increases the nitrogen-retention falls steadily.

These results suggest that, judged by nitrogen-retention, there is an optimum point of protein supply, above or below which nitrogen-retention is reduced. It was realised, however, that this series of observations could not be regarded as in any way conclusive on this point, in view of

¹ *Journ. of Agric. Sci.* **8** (1917), 447.

the varying sources of protein-supply in the different periods, and the fact that the order in which the diverse rations were fed was not that of increasing nitrogen-content. It would be equally legitimate, for example, to account for the reduced nitrogen-retention in Periods III and VI, by assuming the proteins of cottonseed cake and yeast respectively to be of lower metabolic efficiency than those of palm kernel cake.

Both alternatives engaged our interest, as we were at the time contemplating a comprehensive study of the nutritional requirements of the dairy cow, in which, as is well-known, protein-supply plays a part of outstanding importance. It has been established that up to a certain point increased protein consumption leads to increased secretion of milk. Does this point coincide with that of maximum retention of nitrogen in the body? Does the amount of food-protein required to produce the latter vary with the qualitative character of the ration? Is the maximum of nitrogen-retention constant for any one individual or variable according to the feeding? Before attempting to secure answers to these questions we decided first to test whether, by feeding cows on rations of the same qualitative composition, but of successively increasing protein-content, and determining the nitrogen-retention, we could detect an optimum of protein-supply, such as had been suggested by the records of the sheep experiments. Further, in order to avoid the complications that pregnancy and lactation would introduce, we selected as test animals two fully grown Shorthorn cows, not in calf, and not producing milk.

FIRST EXPERIMENT.

(196 days, November, 1916—June, 1917.)

For reasons stated later this experiment cannot be regarded as entirely satisfactory and consequently need only be dealt with in outline.

The two cows were first brought approximately into nitrogenous equilibrium on a daily ration of 14 lb. "seeds" hay, supplying about 122 gm. nitrogen. After determining over a given period the daily balance between the amounts of nitrogen consumed and excreted, the ration was increased by adding 2 lb. maize meal per day (containing about 13 gm. nitrogen) and the effect on the nitrogen balance was then investigated. This was followed by several further experimental periods, in each of which the ration was increased by 2 lb. maize meal.

The general arrangements for the experiment and the methods of analysis, etc., followed were similar to those described in detail later in this paper.

42 *A Study of Nitrogen Metabolism in the Dairy Cow*

In all changes of feeding a transitional period of at least nine days was allowed, whilst the experimental period consisted of at least twelve days, giving a minimum period of three weeks between each change. The essential data obtained are summarised in Table I.

Table I.

Cow A. (Initial weight, 1033 lb.)

Period	Duration days	Daily ration		Total N consumed per day	Gain (+) or Loss (-) of N by cow per day
		Hay lb.	Maize lb.		
I	21	14	0	124.9	- 8.2
II	32	14	2	134.1	- 7.6
III	21	14	4	151.8	+ 18.1
IV	21	14	6	168.4	+ 20.6
V	28	14	8	161.9	- 10.4
VI	21	16.5	8	172.4	- 1.1
VII	28	16.5	10	185.7	- 6.0
VIII	21	16.5	12	201.9	- 17.8
IX	31	16.5	10*	191.3	+ 15.7

Cow B. (Initial weight, 959 lb.)

I	32	14	0	124.3	- 5.0
II	32	14	2	133.3	+ 8.4
III	21	14	4	148.9	+ 18.9
IV	21	14	6	164.3	+ 20.4
V	28	14	8	161.4	+ 18.1
VI	—	—	—	—	—
VII	28	14	10	170.3	+ 21.0
VIII	—	—	—	—	—

* The concentrated food during this period consisted of 9 lb. maize + 1 lb. linseed cake.

The investigation proceeded quite smoothly up to Period V, when it became necessary to draw a fresh consignment of hay. This proved unfortunately to be decidedly poorer in nitrogen than the previous consignment, and consequently, despite the added 2 lb. of maize, the nitrogen consumption for Period V worked out rather less than for Period IV. In order to overcome this difficulty the basal ration of hay was increased for Period VI to 16.5 lb. per day, the maize meal being retained as in Period V at 8 lb. Unfortunately, although Cow A consumed the increased ration of hay satisfactorily, Cow B could not be induced to do so, and in consequence this cow had to be kept for the remainder of the experiment on the lower ration (14 lb.) of hay, so that from this point the records of the two cows were not strictly comparable.

Further difficulty was experienced towards the end of the experiment in inducing the cows to consume the rations completely and it was obvious that we were approaching the limits of their appetites. This was the

more marked with Cow B, and for this reason we were obliged to reject the records of this cow for Periods VI and VIII.

If the data for nitrogen-retention be examined, it will be seen that with each cow up to Period IV there were distinct indications that the daily retention of nitrogen was approaching a maximum value and, but for the regrettable difficulty with the hay, it seems probable that this limit would have been reached in Period V, since in the later periods, despite increased intake of nitrogen, no higher retention than that of Period IV was recorded. A slight increase is indicated with Cow B in Period VII but it will be noted that the nitrogen intake in this case was practically the same as for Cow A in Period IV, so that the maximum retention of nitrogen under the conditions of experiment would seem to be associated with a nitrogen intake of roughly 170 gm. per day (= 1060 gm. crude protein). Taking the average weight of the cows for Period V this represents a daily intake of crude protein of 2.34 kg. per 1000 kg. live-weight, or almost exactly the same figure as was deduced for the sheep from the data obtained in the digestibility trials. This confirmation is particularly interesting in that the diets in the different periods of the sheep tests varied considerably in qualitative character, and the conclusion suggests itself that, when fed along with hay, the proteins of maize, cottonseed, palm kernels and yeast are of equal value so far as capacity to effect protein storage in the body is concerned.

It would appear therefore from these experiments that for the last-named purpose the optimum protein-supply in the food is in the neighbourhood of 2.4 *kg. crude protein*, or say 1.7 *kg. digestible crude protein per 1000 kg. live-weight*. This, of course, applies only to animals such as those under test which were not subject to any losses of nitrogen other than those voided in faeces and urine, and were free from any exceptional internal needs for protein such as arise during pregnancy.

It will be noted from Table I that the duration of the different periods varied from 21 to 32 days. It was anticipated that a period of 21 days would afford ample time for the re-establishment of nitrogen equilibrium after the disturbance due to change of ration. It soon became evident, however, that this was not the case, and the records of Periods IV to VI bring this out clearly. For reasons already given the nitrogen-consumption remained relatively constant throughout these three periods, which may therefore be grouped together as one long period. Viewed in this light it is seen that the establishment of nitrogen equilibrium is a much slower process than we had assumed, the nitrogen-retention persisting, though at a steadily diminishing rate, throughout the whole interval of

44 *A Study of Nitrogen Metabolism in the Dairy Cow*

70 days. In this particular, therefore, our scheme of experiment would appear to have been defective in that the change of ration from period to period was evidently made before the influence of the earlier ration on nitrogen-retention had been exhausted. Accordingly, before proceeding further with the comprehensive programme of experimental work on these lines which lay before us, we thought it desirable to make a more prolonged study of nitrogen-retention under conditions of roughly constant nitrogen-consumption, and a further experiment was undertaken in November, 1917, and developed later into a study of nitrogen-retention during pregnancy and lactation, the whole experiment covering a period of two years.

SECOND EXPERIMENT.

(November, 1917—November, 1919.)

General Plan of Experiment.

Two Shorthorn cows, C and D, similar to those used for the first experiment, both "dry" and not in calf, were weighed and placed on a daily ration consisting of 20 lb. "seeds" hay. The nitrogen-balance in both cows was followed continuously, except for the one week monthly during which the cows were weighed and an interval of 10 weeks at the end of the first year. After a period of 302 days (including 255 days for which the nitrogen-balance records were obtained) during which both cows received identical treatment, Cow D was put to the bull, whilst Cow C remained unserved as control. The nitrogen-balance measurements were continued for both cows throughout the period of pregnancy of Cow D and subsequent parturition, and were further extended well into the period of lactation. For Cow D the experiment extended over periods of 302 days "dry" and not in calf, 284 days "dry" but in calf, and 136 days in milk, a total of 722 days throughout the whole of which period Cow C was maintained dry and not in calf. Samples for nitrogen-balance determinations were taken on 546 days during this period.

Both this and the foregoing experiment were carried out in a building specially designed for work of this character. The floor was of cement, graded and grooved to secure rapid drainage, each cow-stall being provided with a separate drain, so arranged that the drainage could be easily collected for purposes of weighing and sampling. Preliminary tests showed that any liquid falling on the floors of the stalls could be recovered within 2 or 3 per cent., whilst by spraying the floors occasionally with

water a still higher degree of completeness could be obtained in the recovery of urinary nitrogen.

Experimental details.

Collection, Sampling and Analysis of Faeces. The faeces were collected into weighed covered buckets. The 24-hours' collection was weighed, thoroughly mixed, and by quartering, reduced to a small sample. One-tenth of the daily faeces was preserved in a closed vessel with the addition of a little toluene. In this manner, composites were made up twice weekly, representing the collection over three and four days respectively. Preliminary tests had shown that the loss of nitrogen was inappreciable when the faeces were stored in this manner over a period of several days. The composite samples were well mixed and small samples were drawn from the bulk for analysis. The nitrogen-content was determined in triplicate by means of the Kjeldahl method.

Collection, Sampling and Analysis of Urine. The urine was collected in weighed buckets, the drainage being assisted by frequent spraying of the floors with water. No litter was used in the stalls. The daily output of urine was weighed and, after thorough stirring, one-tenth of the total weight was ladled out, acidified with 20 c.c. H_2SO_4 (1 : 1) and preserved in a Winchester bottle. Composites were made up in this way twice weekly and the nitrogen-content of the samples was determined by the Kjeldahl method. Under the conditions of collection the faeces were always contaminated with urine, and the urine was diluted to some extent by the water used in spraying. This was immaterial, however, as it was only required to measure the total voided nitrogen.

Length of periods and weighing of cows. The periods were mainly of about 18 days' duration. At the end of each period the cows were weighed on three successive days under comparable conditions.

Feeding of cows and analysis of foodstuffs. The hay was fed in four equal allowances during the day, care being taken that the cows had consumed the hay completely before being left for the night. Water was given *ad lib.* Composite samples of the feeds were made up by reserving weighed representative samples daily. Two independent hay composite samples were made up in each period, and the dry matter and nitrogen-contents of these were determined, the mean results for the two samples being used in the calculation of the nitrogen-balances. From time to time representative samples were made up from the period composites and complete analyses were carried out. Moisture deter-

46 *A Study of Nitrogen Metabolism in the Dairy Cow*

minations were made daily on independent hay samples, since this factor was found to be subject to considerable variation.

Analysis of milk. Cow D was milked twice daily during its period of lactation and half-weekly composites were made up. The samples were preserved by the addition of one or two drops of formalin. The determination of the nitrogen in the milk samples was carried out in duplicate by the Kjeldahl method.

The experiments were carried out at the Manor Farm, Garforth, (Experimental Farm of the University of Leeds and the Yorkshire Council for Agricultural Education). The care of the animals was in the experienced hands of Mr H. J. Hargraves, N.D.A., to whom we are greatly indebted for the skill and accuracy with which this important side of the work was carried out.

Table II. *Analysis of seeds hay and linseed cake composite.*

(Calculated on dry matter.)

Days represented by sample ...	Seeds hay						Linseed cake
	1-68	69-194	195-295	296-499	500-660	661-722	
Crude protein ...	12.46%	12.41%	11.08%	12.86%	10.27%	10.29%	33.48%
True protein ...	10.25	11.03	9.75	11.45	8.73	9.17	23.86
Ether extract ...	1.52	1.95	1.79	1.65	1.65	1.55	10.15
Nitrogen-free extractives ...	50.23	45.68	51.50	48.50	49.23	49.92	38.95
Crude fibre ...	28.90	33.42	29.64	30.94	31.82	31.22	10.71
Ash ...	6.89	6.54	5.99	6.05	7.03	7.02	7.01

It will be noted that the composition of the hay, and in particular, its content of protein, were subject to considerable variation during the course of the trial. This rendered necessary, in the later stages of the experiment, a slight increase in the amount of hay fed per day, in order to maintain the amount of protein consumed per day at its initial level. Another factor disturbing to some extent and difficult to regulate was the great variation to which the moisture-content of the hay was subject from day to day. For these reasons it was impossible to avoid an appreciable variation in the nitrogen-supply without recourse to methods which the resources at our disposal did not permit. It will be seen from the second column of Table III what the extent of this variation was.

Comments on Table III (First Main Period of Experiment).

We cannot attempt to tabulate here in detail the whole of the data accumulated day by day during the trial. The figures given in Table III therefore represent daily averages for the different periods, the nitrogen-balance columns giving the average daily gain or loss of nitrogen by the

cows for each respective period. The weight columns give the gain or loss of weight of the cows over the whole period.

Table III.

Summary of nitrogen-balances for Cow C and Cow D up to day 295.

Daily ration: 20 lb. seeds hay (average dry matter per day = 7600 gm.).

Period in days	Cow C				Cow D		
	N con- sumed (average per day)	Total N voided (average per day)	Mean nitrogen- balance per day	Change in weight	Total N voided (average per day)	Mean nitrogen- balance per day	Change in weight
	gm.	gm.	gm.	lb.	gm.	gm.	lb.
1- 19	136.0	130.1*	+ 5.9	- 13 $\frac{2}{3}$	132.4*	+ 3.6	- 43
23- 47	151.1	143.7	+ 7.4	- 5 $\frac{2}{3}$	139.7	+ 11.4	+ 15
52- 68	144.5	139.2	+ 5.3	+ 11 $\frac{2}{3}$	136.2	+ 8.3	+ 11 $\frac{1}{3}$
72- 89	152.9	150.3	+ 2.6	- 3 $\frac{1}{3}$	147.7	+ 5.2	- 1
93-110	151.8	150.2	+ 1.6	+ 2	148.2	+ 3.6	+ 12 $\frac{1}{3}$
111-131	148.6	151.2	- 2.6	+ 30 $\frac{1}{3}$	148.9	- 0.3	+ 6 $\frac{2}{3}$
135-152	152.3	149.1	+ 3.2	+ 2	146.8	+ 5.5	+ 15
156-173	148.9	150.9	- 2.0	- 16 $\frac{1}{3}$	145.4	+ 3.5	+ 25 $\frac{2}{3}$
177-194	147.3	147.8	- 0.5	- 3 $\frac{2}{3}$	142.5	+ 4.8	- 14 $\frac{2}{3}$
198-215	165.3	155.6	- 9.7	+ 15 $\frac{1}{3}$	151.8	+ 13.5	+ 19
219-236	141.8	143.0	- 1.2	+ 22 $\frac{2}{3}$	141.0	+ 0.8	+ 33 $\frac{1}{3}$
240-257	136.6	135.0	+ 1.6	+ 7	130.2	+ 6.4	+ 10
261-276	146.5	135.9	+ 10.6	+ 15 $\frac{2}{3}$	135.7	+ 10.8	+ 4
282-295	138.7	134.1	+ 4.6	+ 11 $\frac{1}{3}$	131.0	+ 7.7	+ 24 $\frac{2}{3}$

Condensed summary.

Analytical days	Total N consumed	Total N retained		Nett change of weight	
		Cow C	Cow D	Cow C	Cow D
255	37,616 gm.	866 gm.	1571 gm.	+ 109 lb.	+ 118 $\frac{1}{3}$ lb.

* Includes small amount of nitrogen from milk (cows not quite dry at beginning of trial).

Initial weights of cows 1 Cow C. 1229 lb.
 1 Cow D. 1266 lb.

According to current views on protein metabolism we might expect to find, on introducing a given ration supplying protein in excess of the requirements of the basal metabolism, that nitrogen storage took place at the outset, but that the rate of storage steadily fell until nitrogen-equilibrium was re-established. This process is clearly evident in the records of our two cows. Taking Cow C for example, and overlooking the first 20 days as preliminary, it will be seen that the nitrogen-retention steadily fell and nitrogenous equilibrium was ultimately roughly established, though not until the lapse of about 90 days. Subsequently, between days 93 and 194 the cow was in almost perfect nitrogen-equilibrium, but this was then disturbed by a rise in nitrogen-consumption due to an abrupt rise in the protein-content of the hay. The effect of

48 *A Study of Nitrogen Metabolism in the Dairy Cow*

this seems to have passed away quickly and between days 249 and 257 nitrogen-equilibrium again prevailed. Thus, but for the abnormal period of days 198-215, equilibrium was maintained over a period of 164 days. It was all the more surprising therefore in the subsequent periods from day 261 to day 295 to find that nitrogen storage was again taking place, a phenomenon of which the explanation is not very obvious. It certainly cannot be attributed to more than a small extent to the comparatively small rise in nitrogen-consumption in the period of days 261-278.

The record of Cow D is very similar for the first part of the period, the nitrogen-retention falling steadily until the 100th day or thereabouts when nitrogen-equilibrium was established. The period from the 111th to the 131st day shows an almost perfect nitrogen-balance, but subsequently a curiously persistent small retention of nitrogen was recorded throughout the remaining 161 days, increasing appreciably, as in the case of Cow C, in the last stages of the period.

Over the whole period, out of 37.6 kg. nitrogen consumed by each cow, Cow C retained 866 gm. and Cow D 1571 gm., whilst the gains in live-weight were 109 lb. and 118 lb. respectively.

No clear correlation is evident between the nitrogen balances and the weight changes. For instance, between days 114 and 131, Cow C showed a daily *negative* nitrogen-balance of 2.6 gm. and an *increase* in weight of 30½ lb. for the period. Between days 72 and 89, however, where the cow *gained* 2.6 gm. of nitrogen daily, the weight of the cow suffered a *loss* of 3½ lb. for the period.

The extremely irregular variation of the figures in the nitrogen-balance and weight columns, and the disconcerting rise in the nitrogen-retention after 240 days of relatively uniform feeding, show clearly the danger of placing too much reliance on conclusions drawn from the results of short-period experiments with cattle. A three-weeks' period in work of this character is obviously far too short.

Comments on Table IV (Second Main Period of Experiment).

Cow D was put to the bull on September 6, 1918 (day 303), and the measurements of the nitrogen-balances were suspended until November 8, 1918 (day 366). This break in the measurements was unfortunate, since, on resumption of the trials, distinct indications were obtained that the protein metabolism of Cow D had undergone a marked disturbance in the early stages of pregnancy. Up to day 295, Cow D had shown throughout a uniformly higher positive nitrogen-balance than Cow C. On resumption, however, Cow D was found to be distinctly in

nitrogen deficit, whereas Cow C still retained a positive nitrogen-balance. If the nitrogen-balances throughout were plotted graphically against the days of duration of the trial, it would be found that the curves for the

Table IV.

Summary of nitrogen-balances during period of pregnancy of Cow D.
(Days 366-575.)

Daily ration:

Cow C (to day 558): 20 lb. seeds hay* (average dry matter per day = 7340 gm.).

Cow D (to day 407): 20 lb. seeds hay (average dry matter per day = 7250 gm.).

(Day 408-562): 21 lb. seeds hay (average dry matter per day = 7740 gm.).

Period in days	Cow C				Cow D			
	N con- sumed	Total N voided	Mean daily nitrogen- balance	Change in weight for period†	N con- sumed	Total N voided	Mean daily nitrogen- balance	Change in weight for period‡
	(average per day)	(average per day)	gm.	lb.	(average per day)	(average per day)	gm.	lb.
366-383	143.7	132.8	+ 10.9	- 26 $\frac{1}{2}$	143.7	148.6	- 4.9	- 13 $\frac{2}{3}$
387-407	144.6	131.8	+ 12.8	+ 32	144.6	145.7	- 1.1	+ 12 $\frac{2}{3}$
416-432	136.8	129.9	+ 6.9	- 9 $\frac{1}{2}$	143.6	144.5	- 0.9	+ 25 $\frac{2}{3}$
436-453	138.9	144.1	- 5.2	+ 1 $\frac{1}{2}$	145.8	143.8	+ 2.0	+ 21 $\frac{1}{3}$
457-474	150.2	160.0	- 9.8	- 19 $\frac{4}{3}$	157.7	155.9	+ 1.8	+ 19
478-495	152.6	154.8	- 2.2	+ 5 $\frac{1}{2}$	160.2	155.1	+ 5.1	+ 22
499-523	149.1	161.6	- 12.5	- 18 $\frac{1}{3}$	156.6	154.7	+ 1.9	+ 26 $\frac{1}{3}$
534-558§	145.2	148.4	- 3.2	- 3 $\frac{2}{3}$	145.6	140.8	+ 4.8	+ 10 $\frac{1}{3}$
562-575	146.6	141.6	+ 5.0	+ 20	149.4	131.4	+ 18.0	+ 15 $\frac{2}{3}$

Condensed summary.

Number of analytical days	Cow C			Cow D		
	Total N consumed	Total N retained or lost	Nett change in live- weight	Total N consumed	Total N retained or lost	Nett change in live- weight
	gm.	gm.	lb.	gm.	gm.	lb.
174	25,309	- 50	- 18 $\frac{1}{3}$	24,019	+ 386	+ 139 $\frac{1}{3}$
428	62,925	+ 816	+ 91 $\frac{1}{2}$			

* From day 562 started feeding new consignment of hay. This proved poorer in nitrogen and ration for days 562 to 575 was consequently altered, and average consumption as follows:

Cow C: 21.4 lb. hay + .43 lb. linseed cake (= 8750 gm. dry matter per day).

Cow D: 21.8 lb. hay + .43 lb. linseed cake (= 8920 gm. dry matter per day).

† Weight of Cow C at beginning of period, 1378 lb.

‡ Weight of Cow D at beginning of period, 1407 lb.

§ The records for Cow D in this period relate only to days 534-544. This cow was withdrawn from trial between days 544 and 558 on account of slightly swollen hocks.

|| Does not include weight changes from August 29 to October 31, 1919, during which period no determinations of nitrogen-balance were made.

two cows intersect shortly after service of Cow D; previous to this, the curves run approximately parallel. Thus, on a diet more than sufficient for its protein requirements in the ante-pregnant period, Cow D, as a

50 *A Study of Nitrogen Metabolism in the Dairy Cow*

result of changes in the early stages of pregnancy, was suffering a loss of protein.

This behaviour is in accord with the findings in other investigations carried out on different species. Murlin¹ investigated the weekly nitrogen-balances in a pregnant bitch and showed that there was a large loss of maternal protein, commencing immediately after conception and continuing for six weeks. Only during the last two weeks before parturition was there a marked conservation of protein, as manifested in the pronounced nitrogen-retention. Murlin attributed the destruction of maternal protoplasm which accompanies the development of the foetus to the necessity for providing "hereditary building stones for the laying down of the youthful protoplasm in accordance with the type characteristic of the species."

The diet of Cow D was increased by 1 lb. seeds hay per day on day 408 and later again on day 562 (see Table IV). This slight increase enabled the cow to begin to retain nitrogen. Reference to Tables IV and V shows that the rate of storage, however, was not very considerable until within three or four weeks of parturition. During this time, although the amount of nitrogen excreted in the faeces remained roughly at its former level, yet the nitrogen appearing in the urine underwent a marked diminution. This was a consequence of protein retention. Examining the period as a whole, it would appear that the demands made on the food protein for the single purpose of foetal development were relatively small, since the average rate of nitrogen-retention was only about 2.4 gm. per day.

The behaviour of Cow C, dry and not in calf, during this period contrasted curiously with its behaviour in the preceding period, when it was able to make a distinct gain of protein and body-weight. In this period, though receiving approximately the same ration, Cow C suffered a slight loss of protein and its weight dropped about 18 lb. These results serve to illustrate further the uncertainty of short period work with animals.

Comments on Table V (Third Main Period of the Experiment).

In Table V are given the full experimental details for the period immediately preceding and following the calving of Cow D. Particulars of milk-yield, etc., for this and the following period will be found in Table VIII. For some days after parturition, each day's collections of urine, faeces and milk from Cow D were analysed separately.

¹ *American Journal of Physiology*, 1910, XXVII, 177

Table V. *Nitrogen-balances for Cow D in period of parturition.*

Daily ration: 21·5 lb. of seeds hay + linseed cake (for amounts on different days see Table).

Days of period	Consumed			Nitrogen voided				Daily nitrogen-balance
	Lb. of cake	Total dry matter	Total N	In faeces	In urine	In milk	Total	
				gm.	gm.	gm.		
580-586	1*	9,104	141·8	76·1	50·3	—	126·4	+ 15·4
587	1	9,174	142·5	—	158·6†	51·1	—	(- 99·5
588	1	8,973	140·0	143·7	40·4	87·7	481·5	(- 99·5
				(2 days)				
589	1	9,033	140·8	90·0	61·1	100·0	251·1	- 110·3
590	8	11,826	280·3	98·9	88·2	105·7	292·8	- 12·5
591	8	11,832	280·4	94·6	88·8	76·2	259·6	+ 20·8
592	6	10,960	239·5	114·5	111·1	79·5	305·1	- 65·6
593	6	11,024	240·4	129·3	99·3	81·7	310·3	- 69·9
594	6	11,010	248·1	91·4	125·5	78·2	295·1	- 47·0
595	8	11,718	286·4	100·8	108·2	78·4	287·4	- 1·0
596	8	11,730	286·6	101·9	102·1	76·5	280·5	+ 6·1
597	8	11,830	288·1	103·4	108·0	74·8	286·2	+ 1·9
598-603	8	11,715	286·5	111·6	111·2	69·2	292·0	- 5·5
Average from parturition			252·5	102·2	103·5	76·8	282·5	- 30·0
Average including N of calf and afterbirth ...			252·5	—	—	—	344·3	- 91·8

* 1 lb. linseed cake contained 399·0 gm. dry matter and 19·93 gm. N.

† This figure gives N of urine + fluids + washings. Loss in weight of Cow D during this period was 161 lb.

Cow D calved on the morning of June 17th, 1919 (day 587), the period of gestation being 281 days. The weight of the calf was 84 lb. Estimating the protein-content of the calf at 16 per cent.¹, its nitrogen-content would be 975 gm. The placental fluid, urine and washings were collected together and analysed. The placenta weighed 11·5 lb. and had a nitrogen-content of 74·6 gm. (= 1·43 per cent. N in placenta).

Taking the figure 2·4 gm. as representing the rate of storage of nitrogen per day during the period of pregnancy, it follows that the total storage of nitrogen by Cow D during the period of gestation was approximately:

Up to day 579	665 gm.
From day 580 to parturition	108 ..
Total	773 ..

The losses of nitrogen during parturition were approximately:

In calf	975 gm.
In placenta, etc.	75 ..
Total	1050 ..

¹ Vide Armsby, *The Nutrition of Farm Animals*, p. 62.

52 *A Study of Nitrogen Metabolism in the Dairy Cow*

This figure represents a minimum estimate, since it does not include the nitrogen of the fluids, which were collected with the urine and not analysed separately. Thus, whereas 1050 gm. nitrogen had been stored as tissue in the form of calf and placenta, Cow D had been able, on a ration only very slightly heavier than that which served for its protein requirements when not in calf, to obtain 773 gm. from its food for this purpose. The deficit of 277 gm. must have been supplied from the maternal protein at the average rate of about 1 gm. per day.

A cow in calf is thus able to maintain a positive nitrogen-balance on a ration differing only slightly from that requisite for nitrogenous equilibrium in the ante-pregnant period, though under such circumstances, it may have to supply from its own protein a fraction of the nitrogen necessary for foetal growth, and thus be unable to build up the desirable reserves during pregnancy to enable it to come into lactation with a good milk flow capable of being sustained over a long period. More generous feeding than was given in this investigation is obviously necessary in actual farm practice, especially in view of the heavy negative nitrogen-balances shown by Cow D in the days following parturition.

For the first three days after calving it was thought inadvisable to increase the ration, but on the fourth day (day 590) the allowance of linseed cake was raised to 8 lb. This immediately checked the loss of nitrogen from the body, but as the cow at this stage could barely consume the ration, the allowance of linseed cake was reduced two days later (day 592) to 6 lb., whereupon a marked nitrogen deficit was again established. On day 595 the allowance of linseed cake was again raised to 8 lb. and nitrogen-equilibrium was practically restored, this changing to nitrogen-retention when on day 608 a further 1 lb. of cake was given (*vide* Table VI).

For the period covered by Table V Cow C, dry and not in calf, was again in nitrogen *deficit* (see below) following a short period of nitrogen storage from day 562 to 575.

Daily ration (days 580-603)	N consumed (average per day) gm.	N voided (average per day) gm.	Mean daily nitrogen- balance gm.	Change in weight for period lb.
21.5 lb. hay + 1 lb. linseed cake	143.8	155.6	- 11.8	+ 2½

Comments on Table VI (Fourth Main Period of Experiment).

It will be noted from Tables V and VI that a positive nitrogen-balance was not established in Cow D until three weeks after parturition. It was then consuming daily about 330 gm. nitrogen and was yielding about 27 lb. of milk, containing roughly 60 gm. N per day. That the

ration was then ample for the extra requirements of milk production was evidenced by the consistent positive nitrogen-balances recorded. Towards the end of the trial, when the daily yield of milk had fallen below 20 lb. (containing about 48 gm. N) the amount of cake fed daily was reduced to 5 lb. This resulted in the establishment of a decided negative nitrogen-balance.

Table VI.

Summary of nitrogen-balances for Cow D during period of lactation.

Daily ration: 21.5 lb. seeds hay + linseed cake* (for amounts see Table).

Days of period	Lb. of cake	Consumed (average per day)		N voided (average per day)				Mean daily nitrogen-balance	Change in weight period
		Total dry matter	Total N	In faeces	In urine	In milk	Total		
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	lb.
608-624	9	12,013	329.9	107.2	153.8	59.9	320.9	+ 9.0	- $\frac{2}{3}$
629-645	9	12,039	349.9	109.1	169.2	58.9	337.2	+ 12.7	+ $15\frac{1}{3}$
650-659	10	12,440	340.5	112.8	161.4	57.4	331.6	+ 8.9	- $12\frac{2}{3}$
665-680	9†	11,952	323.5	111.9	143.4	53.6	308.9	+ 14.6	- $33\frac{2}{3}$
685-701	7	11,153	287.7	106.2	124.6	54.7	285.5	+ 2.2	+ 5
706-722	5	10,370	241.3	97.4	106.5	48.7	252.6	- 11.3	- $2\frac{1}{3}$

Condensed Summary for Cow D.

Analytical days	Total N consumed	Total N retained	Nett change in weight
	gm.	gm.	lb.
608-624	29,131	+ 536.8	- 18
546	96,051	+ 2091	+ $78\frac{1}{2}$ ‡
546	96,051	+ 1041§	—

* 1 lb. cake contained 400 gm. dry matter and 21 gm. nitrogen (approx. average for whole lactation period).

† Amount of cake reduced to 9 lb. on day 668.

‡ See note (||) Table IV.

§ Including N of calf and placenta (estimated at minimum of 1050 gm.).

During this period of about 90 analytical days in the lactation period, Cow D actually retained about 537 gm. of nitrogen and only lost 18 lb. body-weight. For the whole trial of 546 experimental days, it will be seen that this cow consumed about 96,000 gm. nitrogen and of this retained 1040 gm. (*i.e.* slightly over 1 per cent.), this being roughly the amount of nitrogen absorbed in foetal development and lost from the body at parturition.

It was originally intended to continue the experiment through the whole period of lactation, but circumstances rendered this impracticable.

It is of interest to compare in the case of Cow D for the period subsequent to calving (Tables V, VI) the consumption of nitrogen over and above "equilibrium requirements" with the amount of nitrogen secreted in the milk. For this purpose we may take the "equilibrium require-

54 *A Study of Nitrogen Metabolism in the Dairy Cow*

ment," judged by the previous records of the cows, at roundly 145 gm. per day.

Day number	Surplus N consumed (total N 145 gm.) (average per day*)	N in milk (average per day*)	Ratio of surplus N to milk N	Nitrogen- balance (average per day)
	gm.	gm.		gm.
590-591	135	91	1.5 : 1	+ 4.1
592-594	98	80	1.2 : 1	- 60.9
595-603	142	72	2.0 : 1	- 2.9
608-645	195	59	3.3 : 1	- 10.8
650-659	195	57	3.4 : 1	+ 8.9
665-680	178	54	3.3 : 1	+ 14.6
685-701	143	55	2.6 : 1	+ 2.2
706-722	96	49	2.0 : 1	- 11.3

* To nearest gm.

It is clearly evident why during days 592-594 a heavy nitrogen deficit was recorded, since the "surplus" nitrogen, even without allowance for digestibility, was barely greater than the nitrogen removed from the body in the milk. Even in the period, days 595-603, when the "surplus" nitrogen was roughly twice that secreted in the milk, equilibrium was barely established, and again later, in the period, days 706-722, when a similar proportion prevailed, nitrogen deficit again set in. In the intervening periods when the proportion was well over 3 : 1 a substantial nitrogen-retention was effected.

It would appear therefore that in order to prevent loss of nitrogen from the body of the lactating cow the "surplus" nitrogen must amount to well over twice the amount of nitrogen secreted in the milk. This is a condition which may well be difficult to satisfy in practice. Cow D was only a moderate milker (about 28 lb. milk daily at maximum), and yet it required the heavy and highly nitrogenous rations indicated to prevent loss of nitrogen from the body. With the more liberal milk-flow so often achieved with the modern dairy cow it would be even more difficult to secure nitrogen-equilibrium, if not indeed beyond the food-consuming capacity of the cow. This is fully in accord with practical experience of the difficulty of maintaining the "condition" of the milch cow in the early stages of lactation.

The full details for Cow C (dry and not in calf) are not given for this period, as they present no new features of interest. The establishment of a positive nitrogen balance is noteworthy, since on the same ration in the preceding period, a decided negative balance was recorded. An examination of the figures for the complete trial shows, however, that Cow C had been able to store about 1000 gm. of nitrogen from the 81,000 gm. consumed.

Table VII.

Condensed summary for Cow C between the days 608 and 722.

Daily ration: 21.5 lb. seeds hay + 1 lb. linseed cake.

Analytical days	Total N consumed	Total N retained	Nett change of weight
	gm.	gm.	lb.
94	14,639	+ 472	+ 13 $\frac{2}{3}$
546	81,015	+ 1005	+ 107

Table VIII. *Milk records of Cow D.*

Days from calving	Daily yield	N	N in milk	Days from calving	Daily yield	N	N in milk
	lb.	%	gm.		lb.	%	gm.
1	3.62	3.110	51.07	53- 56	26.34	0.499	59.58
2	8.75	2.210	87.73	57- 59	25.75	0.497	56.75
3	20.09	1.097	100.00	64- 66	24.80	0.485	54.57
4	28.87	0.807	105.70	67- 70	24.95	0.511	57.83
5	25.56	0.657	76.17	71- 73	25.77	0.510	59.63
6	27.91	0.628	79.52	79- 81	23.25	0.509	53.70
7	28.56	0.631	81.74	82- 84	22.70	0.511	52.40
8	28.93	0.596	78.22	85- 87	22.49	0.534	54.50
9	29.71	0.582	78.44	88- 91	22.27	0.523	53.87
10	29.19	0.578	76.52	92- 94	22.32	0.529	53.57
11	29.87	0.552	74.80	99-101	21.62	0.555	54.40
12-14*	29.56	0.525	70.40	102-105	22.50	0.547	55.85
15-17	28.25	0.531	68.07	106-108	20.92	0.556	52.73
22-24	26.79	0.507	61.63	109-112	22.44	0.545	55.50
25-28	26.85	0.494	60.20	113-115	21.66	0.552	54.17
29-31	27.36	0.472	58.57	120-122	19.37	0.570	50.07
32-35	27.21	0.477	58.88	123-126	19.50	0.558	49.35
36-38	27.35	0.490	60.80	127-129	18.59	0.569	48.00
43-45	26.72	0.479	58.13	130-133	19.28	0.549	48.03
46-49	26.75	0.498	60.45	134-136	18.54	0.575	48.37
50-52	26.14	0.498	59.07				

* From analysis of composite milk samples.

SUMMARY.

This communication deals with the results of two experiments in which the "nitrogen-balance," or difference between nitrogen-consumption and nitrogen-excretion by the cow, has been studied over prolonged periods, starting with the "dry" cow, not in calf, proceeding through the whole period of pregnancy and well into the period of active lactation.

Experiment 1 was performed throughout with the two cows, "dry" and not in calf, receiving a basal ration of hay to which was added increasing amounts of maize meal with a view to securing a progressively increasing consumption of nitrogen. This experiment lasted 196 days, during which period determinations of the nitrogen-balance were made on 90 days.

Experiment 2 was carried out similarly with two cows, and covered a period of 722 days, including 546 days on which determinations of the nitrogen-balance were made. Throughout the whole of this period one

56 *A Study of Nitrogen Metabolism in the Dairy Cow*

cow (Cow C) was maintained "dry" and not in calf, as control cow, whilst the other cow (Cow D) after 302 days became pregnant and its record was followed throughout the stages of pregnancy and parturition and for the first 136 days of active lactation.

The outstanding features of the results are as follows:

(1) With the progressive increase of nitrogen-consumption beyond the fundamental requirements of the dry cow the rate of nitrogen-retention steadily increases to a maximum and then falls. The maximum appears to be attained under the conditions of our experiment with a protein-supply in the neighbourhood of 2.4 kg. crude protein per 1000 kg. live-weight. There are indications that this figure may be independent of the nature of the foods fed along with hay.

(2) When the cow is maintained upon a ration which causes an initial nitrogen-retention the rate of retention falls steadily, but a very prolonged period—up to 90–100 days—may be necessary before nitrogen-equilibrium is attained.

(3) Even after nitrogen-equilibrium is established and a relatively constant nitrogen-consumption is maintained, there may arise from time to time considerable deviations from equilibrium either in the positive or negative direction. It would appear therefore that for reliable work of this character long experimental periods are essential.

(4) The very earliest stages of pregnancy are marked by a profound disturbance of nitrogen metabolism, the requirement for maintenance of nitrogen-equilibrium being very sensibly increased. This additional requirement persists at a steadily reduced rate for some 15 to 20 weeks, after which it is very small. Over the whole period of pregnancy the average rate of nitrogen-retention was only about 2.4 gm. per day.

(5) During parturition and for a few days subsequently the output of nitrogen is very great and more than can be restored rapidly by food-consumption. With the experimental cow, giving barely three gallons of milk per day at most, some two to three weeks elapsed after calving before nitrogen-equilibrium was restored.

(6) It would appear that to maintain nitrogen-equilibrium during lactation, the food must supply from twice to three times the amount of nitrogen secreted in the milk, in addition to that required for the maintenance of equilibrium in the "dry" state. This represents a food-consumption which would be difficult to attain in the case of cows giving large yields of milk, and accounts for the familiar difficulty of maintaining the "condition" of such cows in the earlier stages of lactation.

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THE MENDELIAN INHERITANCE OF SUSCEPTIBILITY AND RESISTANCE TO YELLOW RUST (*PUCCINIA GLUMARUM*, ERIKSS. ET HENN.) IN WHEAT.

By S. F. ARMSTRONG, B.A., F.L.S.

(Cambridge University Plant Breeding Institute.)

CONTENTS.

	PAGE
SECTION I. INTRODUCTION	
(a) <i>Historical</i>	58
(b) <i>Rust scale adopted</i>	61
(c) <i>The soil</i>	61
(d) <i>Weather records</i>	61
(e) <i>The parent wheats</i>	63
SECTION II. THE EXPERIMENTAL RESULTS, 1917-1920	
1. THE F_1 PLANTS, 1917	63
2. THE F_2 GENERATION, 1918	64
(A) <i>Climatic conditions in 1918</i>	64
(B) <i>Spread of rust during 1918</i>	64
(C) <i>Results obtained in the F_2 generation</i>	65
3. THE F_3 CULTURES, 1919	67
(A) <i>Climatic conditions, manuring, and growth of the cultures in 1919</i>	67
(B) <i>Spread of rust during 1919</i>	68
(C) <i>Results obtained from the F_3 cultures</i>	69
4. SUMMARY OF THE F_3 RESULTS	75
5. APPLICATION OF THE F_3 RESULTS TO THE F_2 STATISTICS	76
6. RESULTS OBTAINED FROM F_2 , F_3 , F_4 CULTURES, ETC., IN 1920	78
SECTION III. SOME OF THE FACTORS WHICH MAY INCREASE OR DIMINISH SUSCEPTIBILITY TO RUST ATTACK	
INTRODUCTION	81
(a) EFFECT OF CLIMATIC CONDITIONS	82
(b) FOOD SUPPLY	82
(c) NEW COMBINATIONS OF PARENTAL CHARACTERS	89
(d) VARIATION OF SUSCEPTIBILITY IN HYBRID WHEATS	90
(e) POSSIBLE EFFECT OF ENVIRONMENT UPON THE FUNGUS	91
CONCLUSIONS	92
APPENDIX	94
LIST OF PAPERS REFERRED TO IN THE TEXT	96

SECTION I. INTRODUCTION.

THE present paper may, at first sight, appear to contain much that is irrelevant to the subject as indicated in the title. But, whilst the primary object throughout was to trace the inheritance of susceptibility and resistance to rust attack, experience shows that a study of the environmental conditions is also essential for a correct understanding of this problem.

The unusual weather, and other circumstances in 1919, led to such an abnormal growth of the wheat cultures, that it was felt necessary to present all the available evidence which would throw any light upon the condition of the host plants, the spread of rust, and the greater severity of its attack in that year. For these reasons, details which are usually ignored in the treatment of a subject from the genetic point of view, e.g. such as refer to soil, manuring, and weather, have been included.

(a) Historical.

A large number of investigations have been carried out bearing on the question of the susceptibility and resistance of plants to rust attack, and the more common cereal rusts especially have received much attention. A great deal of this work has been concerned with the histology of the rusts, in which the phenomena of inoculation and infection have been studied under various conditions, and attempts made to discover the primary causes of immunity and susceptibility. Our knowledge of this branch of the subject is based largely upon the investigations of Ward (15-20), Pole Evans (5), Miss Gibson (7), Miss Marryat (10), and Eriksson (4).

The suggestion that immunity depended upon certain slight anatomical differences, e.g. thicker cell walls, fewer or smaller stomata, more or longer hairs, etc., was proved to be incorrect by the researches of Marshall Ward (19) on the Brown Rust (*Puccinia dispersa*) in the genus *Bromus*. Ward showed that the curves of infectibility and those indicating the various anatomical differences, not only failed to correspond, but showed no relation whatever. This led to the conclusion that immunity and susceptibility are not directly determined by the anatomy of the host plant, but depend upon physiological reactions between the protoplasm of the parasite and the cells of the host.

From the experimental evidence obtained, Ward concluded that "infection and resistance to infection depend on the power of the fungus

protoplasm to overcome the resistance of the cells of the host by means of enzymes or toxins, and reciprocally, on that of the protoplasm of the cells of the host to form anti-bodies which destroy such enzymes or toxins" (19). In another place Ward (18) was careful to point out that, "The failure to find any structural or mechanical explanation of the phenomenon (in the sense here implied) does not necessarily involve the assumption that there is no mechanism in the living plant which is answerable for the obstruction, or aid, to infection exhibited by the species. It only points to the conclusion that the mechanism is of that more refined and subtle nature which determines such fundamental properties as specific relationship, variation, heredity, and other biological phenomena."

These quotations from Ward's papers have been made because, in the first place, they represent the final conclusions of an investigator whose work in this direction has never been surpassed; and further, if these conclusions are correct it is reasonable to expect that such features as immunity, etc., should be subject to the general laws of heredity.

Ward (20, p. 37) also inoculated the foliage of Rivet wheat—which is only slightly susceptible to Yellow Rust—with the uredospores of that fungus; at the same time he inoculated a very susceptible variety (Red King), and hybrid plants obtained from a cross between these varieties. Microscopical examination of serial sections showed that inoculation and infection very readily occurred in the very susceptible parent and the hybrids. In the case of the resistant variety (Rivet) it was found that the uredospores germinated, and sent their germ tubes into the stomata as frequently, or nearly so, as they did into the more susceptible wheats. Up to the fourth or fifth day after entry the course of events was also similar, but invariably after this period a process of complete degeneration was observed in the hyphae, and the parasite failed to establish itself. The host cells in the immediate neighbourhood of such hyphae were generally collapsed and their contents disintegrated. From a comparison with experimentally starved hyphae, Ward concluded that the hyphae in this resistant wheat were undergoing death-changes either as the result of starvation or poisoning.

These observations have been confirmed and extended by other workers. Miss Gibson (7) showed that the mere inoculation of the wrong host plants by the uredospores is quite a common occurrence among the Uredineae. She found that in such cases the germ tube enters the stoma as in a normal infection, but that after a period of about four days or less the resulting hypha becomes exhausted and dies. *Her work emphasized*

60 *Mendelian Inheritance and Yellow Rust in Wheat*

the importance of distinguishing between mere inoculation and infection proper, and showed that it is the power of the hyphae to form haustoria which must be taken as the real index of the infective capacity of the rusts. Later, Miss Marryat(10) carried out a very useful comparative histological examination of the foliage of certain immune and susceptible wheats which had been inoculated with Yellow Rust. Her results fully confirmed Ward's previous work, and also clearly proved that different degrees of immunity exist, as is indicated by the relative length of the struggle which goes on between the fungus and the host cells before the former is defeated.

Extensive breeding experiments in recent years have taught us that characters like rust resistance, which are the outcome of complex physiological processes, are nevertheless subject to the laws of Mendelian inheritance. Colour in plants and baking "strength" in wheat are examples of this. With reference to colour, Miss Wheldale(21) says: "There is little doubt that the formation of anthocyanin does involve a series of progressive reactions each of which is controlled by a certain enzyme." Also Keeble and Armstrong(9) present what they believe to be convincing evidence in favour of the hypothesis "that pigmentation is the outcome of the action of oxydase on chromogen." Yet, in spite of the complexity of the reactions involved, the appearance of definite colours has been shown by numerous experiments to obey the Mendelian laws.

The foundations of our knowledge concerning the inheritance of rust resistance were laid some years ago by Biffen(1, 2 and 3) in England, and Nilsson-Ehle(11) in Sweden. Biffen's experiments showed that resistance to Yellow Rust was inherited as a simple Mendelian recessive character. Thus, in a cross between a susceptible and an immune variety, the hybrids were all as badly rusted as the susceptible parent. In the F_2 generation one-quarter of the plants were highly resistant or immune, while the remainder were rusted to various extents. Unfortunately, in the F_3 generation many of Biffen's cultures suffered from various causes, and the mortality was so high as to render the results inconclusive in certain respects. It was clear that rust-free F_2 plants produced only rust-free individuals in the next generation, and also that some of the susceptible F_2 's gave rise to susceptible plants only. But there still remained considerable doubt as to the behaviour of the progeny of the susceptible F_2 plants taken as a whole. The primary object of the work here described was to clear up the points of uncertainty just referred to. It was decided to go over the whole ground from the beginning, and,

if possible, to carry the experiments out on a larger scale, and over a longer period than had previously been done.

(b) *Rust scale adopted.*

In recording the severity of rust attack, an arbitrary scale was employed similar to that used by Eriksson(4) and Biffen(2). In the present case the relative extent of attack was indicated as follows: 0 = a rust-free plant; 1 = a slight attack; 2 = a moderate attack; 3 = a bad attack; and 4 = a very severe attack. When examining the F_2 crop, it became apparent that a considerable proportion of the plants, though not completely rust-free, showed only the merest traces of attack. It was thought advisable to distinguish these from the distinctly rusted individuals grouped under grade 1, and they were therefore given a special mark (1r).

Such a scale, of course, can only indicate the *relative extent* of rust attack in a given season. In an exceptionally bad rust year (e.g. 1919), plants placed in grade 3 may be as severely attacked as those which are placed in grade 4 in another season; and similar seasonal fluctuations may occur among the other grades. But, although the method is not perfect, it appears to be the only practical one that can be used under field conditions where thousands of plants have to be examined in a comparatively short time. Further, this scale has been found sufficiently reliable for the purpose in view, and it has therefore been adhered to throughout the whole of these investigations.

(c) *The soil.*

The soil in the cages (on which all the cultures were grown up to the end of 1919) may be described as a medium gravelly loam. A sample of the top soil to a depth of seven inches was taken in September 1919, and the analysis showed that it contained an ample supply of plant food materials in an available form.

Apart from a light top-dressing of superphosphate applied in the spring of 1917, it had received no manure since 1910. Since the last-mentioned year it had been hand-dug, and always carried a cereal crop.

(d) *Weather records.*

Records of the weather are given in Table I. These were taken at the University Botanic Garden about a mile and a half distant. In addition, daily notes were taken of the weather conditions at the University Farm.

62 *Mendelian Inheritance and Yellow Rust in Wheat*

Table I. *Rainfall, Sunshine, and Mean Temperature at Cambridge from January to July for the years 1917 to 1920.*

Year and months	Total rain inches	Average daily:	
		Sunshine hours	Mean temperature Centigrade degrees
1917			
January to March	3.83	1.08	1.9
April 	1.52	4.27	5.2
May 	1.36	7.39	14.0
June 	1.95	7.20	16.6
July 	4.46	7.39	16.5
Total ...	13.12		
1918			
January to March	3.65	2.89	5.2
April 	3.26	3.00	6.6
May 	2.16	6.84	13.0
June 	1.20	7.10	13.2
July 	2.56	6.39	16.2
Total ...	12.83		
1919			
January to March	2.88	1.81	2.5
April 	2.57	3.77	6.7
May 	0.23	8.35	12.9
June 	1.41	7.73	14.2
July 	3.08	3.55	13.9
Total ...	10.17		
1920			
January to March	3.96	3.10	6.1
April 	3.44	2.90	9.1
May 	1.16	7.84	12.7
June 	1.24	7.73	14.9
July 	2.91	5.29	14.5
Total ...	13.01		

Instead of looking merely at the monthly totals or averages, it appears better to consider separately the more or less distinct "weather periods" into which each season was naturally divided. This enables one to appreciate better the effect of the weather upon the growth of the host plants, and also its effect upon the general prevalence and spread of rust. The weather is thus briefly described under each season in Section II.

(e) *The parent wheats.*

The varieties of wheat chosen for the main experiment were *Wilhelmina* and *American Club*. It is of interest to note that these varieties differ in several important respects, e.g.:

<i>Wilhelmina</i>	<i>American Club</i>
Beardless ear	Bearded ear
Medium-lax ear	Dense ear
White chaff	Red chaff
Medium-short straw	Long straw
Starchy grain	Flinty grain.

Under equal conditions *American Club* also matures earlier than *Wilhelmina*. The main point in this connection, however, is that under normal conditions *American Club* is immune to Yellow Rust, whilst the other variety is moderately susceptible.

Both varieties are susceptible to the Brown Rust (*P. triticina*, Erikss.), but, fortunately, this species did not interfere with the observations on Yellow Rust. Brown Rust does not usually make its appearance in the uredo stage in the Cambridge district before the middle of June. Again, so long as the host remains green there is no difficulty in distinguishing the one rust from the other. Occasionally, when the foliage of a plant is beginning to shrivel up, some doubt may occur; but such cases are rare.

In June 1916, the cross (No. 120) *Wilhelmina* ♀ *American Club* ♂ was made. About 20 grains were obtained, and these were planted in the breeding cage on September 30th.

SECTION II. THE EXPERIMENTAL RESULTS, 1917—1920.

(1) THE F_1 PLANTS, 1917.

The F_1 plants had ample space and grew vigorously during 1917, a season that was remarkable for the comparative mildness of the rust attack in the Cambridge district.

Hot weather was general from May until the end of July, and the rainfall was moderate except during July, which was a very wet month (see Table I). The F_1 plants had a moderate attack of Yellow Rust, and were harvested about the middle of August.

A portion of the grain from these plants was sown on November 9th in rows numbered 1 to 42. In the following year on February 26th a further sowing was made in rows numbered 43 to 82. The object in making two sowings was partly to lengthen the period during which

64 *Mendelian Inheritance and Yellow Rust in Wheat*

observations could be made in 1918, and partly to see whether any difference in respect of the severity of attack would occur on the more advanced and less advanced plants respectively. A portion of the grain from the F_1 plants was saved for sowing in successive seasons, and in this way F_2 generations from the same hybrids were raised in 1918, 1919 and 1920.

(2) THE F_2 GENERATION, 1918.

(A) *Climatic conditions in 1918.*

Monthly records of the rainfall, etc., during the growing season are given in Table 1. The following brief description is taken from daily observations, and will serve to indicate the general character of the season.

The month of April was very wet, with a high average percentage of atmospheric humidity, and less than the normal amount of sunshine. During May the weather was normal, and the wheats made steady progress. May 3rd to 22nd was a warm period during which some heavy showers fell; this period ended with a thunderstorm and heavy rain on the 23rd. This was followed by a fine warm period which lasted until June 17th. By this date the plants were beginning to need rain, but they had not been checked in any way.

From June 18th to 25th the weather was for the most part cool and wet. Warm weather followed from June 26th to July 8th, and then came a period of dull, mild, wet weather which lasted until July 28th. July 29th to August 16th was a fine, warm period.

It will be seen that the season was divided into several comparatively short alternating periods of fine and wet weather. This led to a steady and normal growth of the plants and they were harvested in good condition during the last-mentioned period.

(B) *Spread of rust during 1918.*

Pustules of Yellow Rust were first seen on April 8th, and by May 18th its spread had become general; but the intensity of its attack was still of a slight nature. It continued to spread very gradually until the end of May, but so slow was its progress that at this date it was feared the season might prove unsuitable for reliable statistics to be taken of the F_2 crop. Towards the end of May, however, it began to spread very rapidly, and by June 21st this rust was very prevalent throughout the cages. By the last-mentioned date the foliage of the more susceptible plants was clothed with pustules.

The following records illustrate the progress of the rust attack during 1918:

The autumn sown portion of the F_2 crop contained 829 plants, of which 627 were finally rusted. Of these "finally rusted" individuals, 54 per cent. were attacked by June 10th to 13th; 90 per cent. by June 29th; and 100 per cent. by July 30th.

The progress of the rust attack in 1918 is further illustrated by the notes on the two varieties given below.

Wilhelmina (61 plants).

May 18th, a little rust seen.

June 1st, 23 per cent. of the plants slightly rusted.

June 8th, 81 per cent. of the plants rusted.

June 21st, 100 per cent. of the plants rusted.

Extra Squarehead II (29 plants).

April 8th, one pustule on the culture.

May 18th, a few plants slightly rusted.

June 1st, 34 per cent. of the plants slightly rusted.

June 8th, 65 per cent. rusted.

June 21st, 100 per cent. rusted.

These observations show that Yellow Rust spread most rapidly in 1918 during the last week in May and the first three weeks in June.

(C) *Results obtained in the F_2 generation (1918).*

From the middle of March onwards all the F_2 plants and the parent cultures were kept under observation. By June 8th over 80 per cent. of the plants in an adjacent plot of *Wilhelmina* wheat were rusted, and it was therefore decided to start at once a systematic examination of the F_2 plants.

The method of recording results was as follows:

Each plant received a number to indicate the number of the row and also its position in the row; e.g. 40/10 referred to the tenth plant in the fortieth row, and so on. This made all subsequent references easy. Each plant was then carefully examined leaf by leaf, and the presence or absence of rust attack entered in a note-book according to the previously mentioned scale.

The autumn sown plants were examined in this way at three different periods, viz. First, from June 10th to 13th; again from June 29th to July 3rd; and lastly, from July 30th to August 3rd.

66 *Mendelian Inheritance and Yellow Rust in Wheat*

Rows 13 to 82 (sown February 1918) were examined at two different periods, viz. June 15th to 17th, and again July 16th to 29th.

By the end of July the plants were beginning to mature, and, after making the final examination of the autumn-sown crop, it was found that the spring-sown portion was too far advanced for another examination to be made. In each case the final examination was made only a few days previous to the ripening of the plants.

Table II. *Results from grading the F_2 plants (1918) according to the extent of the rust attack. (Cross No. 120.)*

Date of sowing	Total No. of plants	Extent of the rust attack					
		None	Trace	Slight	Moderate	Bad	Very severe
Autumn: Nov. 9th, 1917	829	202	69	210	162	117	69
Spring: Feb. 26th, 1918	731	145	98	181	132	89	86
Totals	1560	347	167	391	294	206	155

The final condition of the F_2 generation is shown by Table II. Of 829 plants in the autumn-sown portion, 202 remained rust-free throughout the season, while the remaining 627 plants showed evidence of more or less susceptibility. This is a close approximation to the 3 : 1 Mendelian ratio.

In the spring-sown portion, it will be observed, the proportion of completely rust-free individuals was considerably less than one-fourth of the total number, but, on the other hand, the proportion of plants bearing only traces of rust was much higher than in the autumn-sown crop.

The whole F_2 generation contained 1213 rusted and 347 rust-free individuals. If the badly rusted plants (grades 3 and 4) be separated from those that were less severely attacked, we obtain the following totals:

Attack, bad to very severe	361 plants
Attack, moderate or less	852 plants
Entirely free from rust	347 plants

While these figures do not show a very close approximation to the simple Mendelian ratio, they are certainly very suggestive of it. Further consideration of these figures is deferred until the later results have been given.

(3) THE F_3 CULTURES, 1919.

Since it was impossible to deal with sufficiently large F_3 cultures raised from all the F_2 plants, a number of the latter were chosen so as to include individuals showing every grade of attack, as well as a number of those that had remained rust-free. Altogether 198 F_2 plants were taken, and the grain sown in the autumn of 1918.

(A) *Climatic conditions, manuring, and growth of the cultures in 1919.*

Monthly records of the weather are given in Table I. The season proved a most trying one for the plants, there being three prolonged periods of extreme climatic conditions.

The first of these was from January 18th to February 13th, during which the ground was frozen and snow-covered. So unfavourable was the spring weather that as late as the beginning of May the cultures were still in a very backward condition. Before they had sufficient time to recover they were faced by a period of drought which lasted from May 13th until June 19th. Throughout this period of 38 days the average daily sunshine was 10.1 hours, but the total rainfall only 6.2 mm. Indeed the total fall of rain from May 1st until June 19th amounted to only 8.9 mm., i.e. 0.35 inch during 50 days.

On May 27th a top-dressing of nitrate of soda was applied to all the cultures, at the rate of 4 cwt. per acre. It was hoped that this heavy application would counteract the drought effects to some extent as soon as rain fell. However, rain did not fall in sufficient quantity to carry the nitrate into the soil until 23 days later. By June 17th it appeared probable that most of the cultures would be ruined, as many of the plants were in a critical condition and the basal foliage was dying off. Three days later (June 20th) a heavy fall of rain carried the nitrate to the roots. The change was sudden and most marked, for by June 23rd most of the plants showed a notable recovery, and soon afterwards they began to send up numerous side tillers.

The period of drought referred to was immediately followed by a lengthy period of the very opposite conditions. From June 20th until the end of July the weather was cool, dull, and moist. During this time (42 days) 4.4 inches of rain fell, but the average daily sunshine was only 3.6 hours. One of the general effects of this weather—combined with the action of the nitrate—was to delay greatly the ripening of the plants.

68 *Mendelian Inheritance and Yellow Rust in Wheat*

(B) *Spread of rust during 1919.*

Yellow Rust was observed for the first time on May 8th, a month later than in the previous year. During May, and up to about the 20th of June, its spread was very slow, except in the case of very susceptible varieties and cultures. This difference in regard to the "period of infection" was very remarkable. For example, a variety of wheat which may be referred to as "B" was grown alongside the F_3 cultures, and proved to be extremely susceptible to Yellow Rust. Several of the plants were rusted by May 16th, i.e. fairly early in the period of drought. By May 24th almost every plant bore numerous pustules, and on the 31st of May, out of 136 plants in the plot, 135 were excessively rusted. A large plot of another variety (Sudanese wheat) was grown in the neighbourhood, and this was also attacked very early by Yellow Rust. This attack was so severe that, by the middle of June, all the plants were prematurely destroyed, and no grain was formed.

These cases are here referred to because *the attacks developed with great intensity during a period of drought*, and afforded a striking contrast to the scarcely perceptible attacks made at the same time on moderately susceptible varieties. They demonstrate that the more susceptible varieties are liable to an *earlier* successful attack than less susceptible kinds; and also that, on very susceptible wheats at least, Yellow Rust is quite capable of making rapid progress in the tissues of the host during the hottest weather we are likely to experience in this country.

Similar differences as regards the period of infection were observed among the F_3 cultures. By June 11th rust was spreading rapidly on all those cultures which finally proved to be pure susceptibles; on the others, there was either no rust at all, or its progress was very slow (see Table XI).

In order that all the plants might be exposed to an equal chance of infection, on the evening of June 13th all the cultures were sprayed with water teeming with fresh uredospores of Yellow Rust obtained from a neighbouring crop. This procedure was probably quite unnecessary, since the pure susceptible cultures (already containing many attacked plants) were well scattered over the entire experimental area. Almost immediately after the fall of rain on June 20th, a distinct increase in the rate of rust-spread was noticed, and during the cool, moist weather which prevailed in July the attack developed into an epidemic of the greatest severity the writer has ever seen.

(C) *Results obtained from the F_3 cultures (1919).*

(a) *General.* The spring drought proved a very severe test for all the cultures, and some of them, which were on rather poorer ground, had to be abandoned. Ample material was provided by the remaining 170 cultures which contained some 8500 plants. These cultures were examined in regular order, and the first appearance of rust on each noted. Immediately an attack was observed, all the plants were numbered, and the rusted ones indicated in a book. The cultures were repeatedly examined, from the first week in May until the end of July or later, to see to what extent the attack had spread. *At the last examination the intensity of attack on each plant was noted as in 1918.*

The conditions under which the plants were grown proved so favourable to rust attack that, almost without exception, the cultures were more severely rusted than their F_2 parents had been in the previous year. This is indicated to some extent by the results given in Tables III to VII. Cultures raised from badly rusted F_2 plants were still more severely attacked in 1919. It will be seen also that, in the 69 cultures which gave evidence of segregation, very few plants actually remained rust-free.

Table III. *Results of analysis of the F_3 cultures (1919)
grown from rust-free F_2 plants.*

Culture No.	Plants in culture	Extent of the rust attack at final examination (28th July to 11th August)					
		None	Trace	Slight	Moderate	Bad	Very severe
66/9	49	49	—	—	—	—	—
11/21	28	28	—	—	—	—	—
2/10	45	44	1	—	—	—	—
19/12	46	40	6	—	—	—	—
2/18	41	27	6	12	2	—	—
82/14	25	1	11	10	—	—	—
8/17	60	25	6	20	15	—	—
68/19	42	13	6	18	4	1	—
29/15	43	16	6	11	14	2	—
57/8	62	6	11	41	10	—	—
Totals	441	243	38	112	45	3	—
26/22	7	6	1	4	2	—	—
28/24	42	6	7	25	8	2	—
3/1	51	7	6	19	25	—	—
15/5	34	6	1	18	8	7	—
24/3	19	1	6	4	11	3	—
25/9	29	—	—	—	11	16	2
22/27	30	—	—	—	11	14	5

(b) *Cultures raised from rust-free F_2 plants.* Seventeen of these cultures were grown, but in only two of them did every plant remain absolutely free from attack. Of these seventeen cultures, the first ten in Table III

70 *Mendelian Inheritance and Yellow Rust in Wheat*

suffered least from drought, and their growth was almost normal; if we limit our attention for the moment to these we note the following facts: (1) they all remained perfectly free from rust up to about July 8th (see Table XI); (2) cultures 66/9 and 11/21 were entirely rust-free until maturity; and (3) the other eight cultures each contained some plants that were attacked to various extents. In most cases these attacks were of a slight character only, though three plants out of a total of 361 had actually bad attacks. Careful observation showed that the parasite did not thrive on the plants in these cultures, except in the rare cases just noted. The pustules, though often numerous, were abnormally small, and a large proportion of them failed to burst the epidermis, or only did so as the foliage was beginning to shrivel up. Owing to the difficulty in making due allowance for the milder nature of the attack, as distinct from the relative number of pustules borne, it is probable that the susceptibility of some of these plants has been overstated by the grades accorded to them. Moreover, a comparison of these cultures with the homozygous susceptible ones growing alongside showed that the *relative difference* in the extent of rust attack was as great as had existed between their respective F_2 parents in the previous season.

Cultures 26/22, 28/24, 3/1, 15/5, and 24/3 are grouped by themselves in Table III because they were much affected by drought, and their growth was far from normal. Although finally rusted to a greater extent than the first ten cultures, like them they remained uninfected till as late as July 8th, and the evidence strongly suggests that they were also derived from "genetically immune" F_2 's.

It was obvious that the extent to which the power of resistance was disturbed varied largely from culture to culture, and also among plants in the same culture. Further, the greatest "disturbance" occurred in those cultures that were most affected by drought, and possibly this may also partly explain why the attack varied so widely on different individuals. Although it is impossible to state the exact extent of this "disturbance," from the evidence obtained it seems safe to conclude that it was sufficient to allow "genetically immune" plants to become either slightly or in some cases moderately attacked. This must be borne in mind when considering the remaining F_3 results.

Biffen (3, p. 123) found in his experiments that the immune F_2 plants bred true to that character; and in the present case, when all the experimental evidence was taken into consideration, the only conclusion one could arrive at was that these fifteen cultures were "immune" in the genetic sense, although in many instances the plant's power to resist

attack had been modified or partially broken down. This greater or less degree of predisposition to attack was probably due to the interaction of other causes or "factors" such as are discussed later under section III.

The last two cultures in Table III (25/9 and 22/27) were attacked considerably beyond the average, though not nearly so severely as the pure susceptible cultures were. They were probably the progeny of heterozygous susceptible plants that escaped infection in 1918.

(c) Twelve cultures (Table IV) were raised from F_2 plants which showed only traces of attack. Three of these proved highly resistant and compared closely in this respect with the first fifteen cultures given in Table III. One of these (82/9) was further tested on a large scale; forty-seven F_4 cultures were grown containing some 850 plants, all of which proved highly resistant in 1920. It is therefore reasonable to conclude that the two cultures, 31/23 and 70/13, were also the offspring of homozygous "immune" plants, although these plants showed traces of attack in 1918.

Table IV. *Results of analysis of F_3 cultures (1919) grown from F_2 plants which had only traces of rust attack in the previous season.*

Culture No.	Plants in culture	Extent of the rust attack at final examination (28th July to 11th August)					
		None	Trace	Slight	Moderate	Bad	Very severe
12/20	48	—	—	1	9	17	21
14/18	54	—	—	1	29	14	7
9/6	53	—	—	6	32	9	6
16/15	67	—	—	2	51	12	2
10/10	26	—	—	3	14	9	—
72/7	53	1	—	21	21	9	1
32/1	40	9	0	11	14	5	1
66/1	63	20	8	13	14	7	1
41/17	(44)	—	—	—	—	—	—
Totals:	404	30	8	61	184	82	39
[8 cultures]		99			305		
31/23	48	4	—	24	20	—	—
70/13	44	2	7	30	5	—	—
82/9	61	—	10	49	2	—	—

The other nine cultures gave evidence of segregation into slightly, moderately, and badly rusted types, and were clearly the offspring of heterozygotes for rust resistance. In eight of these the plants were fully graded and included 99 individuals with a slight attack, or none, and 305 with a moderate or bad attack.

(d) Of the cultures raised from only slightly rusted F_2 's, 26 gave distinct evidence of segregation (Table V). Twenty-two of these plants

72 *Mendelian Inheritance and Yellow Rust in Wheat*

were graded at the final examination, and these contained 291 individuals with only a slight attack or none, and 893 on which the attack was either moderate, bad, or very severe. These numbers are very close to the 3 : 1 Mendelian ratio. Of the other cultures in this group, seven showed much less evidence of segregation, but, as the majority of the plants in each case were much less severely attacked than those of the homozygous susceptible cultures growing alongside, it is probable that these were the offspring of heterozygotes.

Two other cultures, 1/3 and 38/22, proved to be as resistant as the first 15 given in Table III.

Table V. *Analysis of F_3 cultures (1919) grown from F_2 plants which had a slight attack of rust in 1918.*

Culture No.	Plants in culture	Extent of the rust attack at final examination (28th July to 11th August)					
		None	Trace	Slight	Moderate	Bad	Very severe
2/8	52	12	—	9	20	8	3
7/15	48	11	—	9	5	12	1
9/3	70	15	—	22	28	4	1
11/3	51	11	—	7	26	5	2
67/10	54	12	—	6	10	11	15
67/11	73	11	3	12	24	8	15
13/13	19	21	0	9	9	8	2
39/7	87	—	—	15	48	14	10
41/16	48	—	—	8	28	6	6
52/13	48	—	—	8	24	10	6
52/23	67	—	—	13	31	17	6
56/14	65	—	—	20	21	6	18
72/2	44	—	—	5	13	7	19
72/12	50	—	—	3	15	12	20
9/18	29	—	—	7	12	9	1
10/9	49	—	—	5	23	15	6
17/13	66	—	—	20	42	4	0
17/24	62	—	—	10	43	7	2
22/1	48	—	—	2	30	16	0
23/17	50	—	—	3	21	25	1
24/15	31	—	—	1	10	18	2
8/5	43	—	—	1	28	10	4
30/11	(63)	—	—	—	—	—	—
32/16	(34)	—	—	—	—	—	—
33/23	(44)	—	—	—	—	—	—
38/6	(32)	—	—	—	—	—	—
Totals:	1184	93	3	195	511	238	144
[22 cultures]		291			893		

(c) Sixty-three cultures were raised from F_2 plants which had been moderately attacked. Fourteen of these proved to be homozygous susceptibles, for every plant in these 11 cultures (791 plants in all) was either badly or very severely rusted.

Thirty-one cultures gave clear evidence of segregation, though only nine of these actually contained any rust-free plants at maturity. Details of rust attack on each plant in 24 of these cultures are given in Table VI. It will be noted that the extent of "disturbance of resistance" varied considerably from culture to culture. In the first four given in this table, it was practically non-existent; in the next four it was appreciable, while in most of the others it was very great. Nevertheless, the very wide difference between the nature of the attack in the extreme grades, and the proportion of cases of intermediate attack, indicated that Mendelian segregation had occurred in all these cultures.

Table VI. *Results of analysis of the F_3 cultures (1919) raised from F_2 plants which had a moderate rust attack in 1918. In this table are grouped 24 cultures which gave clear evidence of segregation.*

Culture No.	Plants in culture	Extent of the rust attack at final examination (28th July to 14th August)					
		None	Trace	Slight	Moderate	Bad	Very severe
68/10	65	15	2	1	10	12	25
1/15	42	11	—	25	5	1	0
14/6	32	8	—	1	18	4	1
67/9	57	10	1	5	6	9	26
55/2	119	5	—	17	53	19	25
68/5	39	4	—	2	6	11	16
71/14	52	1	1	26	14	7	3
4/1	112	1	—	23	63	18	7
57/2	64	—	—	3	16	43	2
2/9	42	—	—	3	27	8	4
8/13	40	—	—	2	26	9	3
8/15	71	—	—	13	40	15	3
11/10	31	—	—	1	18	10	5
12/3	52	—	—	2	23	20	7
18/2	48	—	—	3	31	8	3
18/4	55	—	—	1	17	33	4
28/13	40	—	—	8	9	15	8
40/5	46	—	—	4	21	14	4
40/17	44	—	—	16	25	2	1
54/4	78	—	—	5	55	16	2
24/18	39	—	—	2	2	15	20
73/8	76	—	—	4	23	20	29
77/10	57	—	—	15	14	16	12
78/2	55	—	—	9	21	13	12
Totals:	1359	55	4	191	549	338	222
		250 (339.7)			1109 (1019.3)		

In addition to the above, seven other cultures gave clear evidence of segregation, but full records of rust on each plant were not made.

Most of the remaining 18 cultures had suffered very severely from drought. In these segregation was much less evident, the attack varying from a moderate to a very severe one. They were, however, distinctly

74 *Mendelian Inheritance and Yellow Rust in Wheat*

intermediate between the homozygous susceptible and "immune" cultures in regard to their rusted condition, and, taking their abnormal growth into consideration, one is probably correct in concluding that they were the offspring of F_2 heterozygotes.

(f) Nineteen cultures were grown from F_2 plants which had been badly rusted in 1918. Fifteen of these proved to be homozygous susceptibles, every plant being either badly or very severely attacked. One culture (11/2) was seriously affected by drought, but was probably also of the same genetic constitution for susceptibility. The three other cultures, 71/12, 6/1, and 31/1, gave clear evidence of segregation.

(g) The 24 cultures raised from very badly rusted F_2 plants all proved to be homozygous susceptibles. In each of these all the plants were distinctly worse attacked than their parents had been in the previous season (Table VII).

Table VII. *Results from 24 F_3 cultures (1919) raised from F_2 plants which were very badly rusted in 1918. Every plant in this group of cultures was very severely rusted in 1919.*

Culture No.	Plants in culture	Rust first seen	Percentage of plants rusted			
			May 16th to 31st	June 10th to 18th	July 4th to 10th	July 28th to Aug. 9th
9/19	33	May 28	6	33	91	100
11/21	31	" 16	6	82	100	100
27/21	51	" 17	4	88	100	100
28/11	33	" 17	3	81	100	100
29/6	21	" 17	5	72	100	100
31/7	44	" 17	2	20	75	100
33/2	23	" 29	12	52	83	100
34/13	24	" 28	12	63	100	100
42/5	33	" 31	3	51	93	100
51/16	20	" 29	10	45	85	100
55/17	47	" 29	13	49	98	100
57/3	62	" 19	4	50	97	100
64/20	24	" 27	8	50	100	100
68/12	41	" 29	4	57	98	100
75/11	26	" 22	8	92	100	100
80/11	15	" 27	20	73	100	100
16 cultures	537	Average %	7.3	59.8	94.9	100
29/2	22	May 28				100
41/5	25	" 29				100
15/7	34	" 16				100
16/2	32	" 28				100
8/16	34	" 28				100
46/20	23	" 19				100
3/11	30	June 7	—	—		100
81/12	20	" 7	—	—	—	100
24 cultures	757					

(h) *Order of "first appearance" of rust on the different cultures.* It is interesting to notice the order in which the F_3 cultures became infected. This is shown in Table XI. As early as May 17th, 13.2 per cent. of the pure susceptible cultures were infected. At that date, none of the homozygous "immune" cultures, and only 3.4 per cent. of those in which segregation occurred, contained any infected plants. By June 14th, all the pure susceptible cultures were badly attacked, but as yet no sign of infection was observed on the homozygous "immune" cultures, while an intermediate percentage of the segregating cultures were infected. By July 12th, 93.7 per cent. of the last-mentioned cultures were attacked, but only a trace of rust was then present on one plant in all the homozygous "immune" cultures. These results are in the order of expectation if susceptibility and immunity are inherited in simple Mendelian fashion.

The fact that the F_3 cultures were of three kinds was emphasized, not only by the "dates of first infection," but also by the rate at which rust spread throughout the cultures. There was not sufficient time to go fully into this matter, but the figures given in Table VII will serve to indicate how rapidly the infection spread among the plants in the pure susceptible cultures. In these, the great majority of the plants were very badly rusted by July 1th to 10th, whereas no rust was to be found on the pure "immune" cultures at that time. Although full statistics were not obtained from the segregating cultures, it was clear that the rate of rust spread was of an intermediate character in these.

1. SUMMARY OF THE F_3 RESULTS.

The general results obtained from the F_3 cultures may be briefly summarized as follows:

(A) Up to as late as July 10th the cultures were sharply divided into three groups:

1. Those in which every plant was severely attacked.
2. Those in which no trace of attack was to be found.
3. Those in which the extent of attack varied very markedly from plant to plant, some of the plants being rust-free.

(B) The homozygous susceptible (1.) cultures were all characterized by:

- (a) A comparatively early infection.
- (b) A very rapid spread of the disease.
- (c) An exceptionally severe attack on every plant.

76 *Mendelian Inheritance and Yellow Rust in Wheat*

(C) The homozygous "immune" cultures (2.) were characterized by:

(a) Remarkable resistance to attack under the most adverse external conditions.

(b) Extreme lateness of infection where it occurred.

(c) The comparatively mild nature of the attack (and in some cases its complete absence).

(D) The cultures in which segregation occurred (3.) took generally an intermediate position as regards the period of infection and rate of rust spread; though finally a proportion of the plants were as severely rusted as those in the pure susceptible cultures.

The 56 cultures in which segregation was clear, and for which full statistics are available, contained 3045 plants, of which 2385 were either moderately or badly rusted, while 660 had only a slight attack or none. The numbers 2284 : 761 would exactly represent the 3 : 1 Mendelian ratio, and if it exists here it follows that 101 plants out of a probable 761 recessives were rusted beyond the slight extent indicated by grade 1, i.e. 13.2 per cent. But this degree of "disturbance in rust resistance" is quite comparable with that which occurred in the 15 homozygous "immune" cultures given in Table III where, out of a total of 594 plants, 144, or 19 per cent., were rusted beyond grade 1. Further, since the F_3 results have shown that these 15 cultures were the progeny of genetically immune parents, there is sufficient evidence to show that such genetically immune plants may under very adverse conditions be subject to a mild attack. This being the case, it appears safe to conclude that in the above-mentioned segregating cultures one-quarter of the plants were genetically immune, and that these cultures were the product of F_2 heterozygotes for rust resistance.

5. APPLICATION OF THE F_3 RESULTS TO THE F_2 STATISTICS.

Table VIII gives a summary of the results obtained from all the F_3 cultures. It will be seen that it is comparatively easy to pick out homozygous susceptible and immune types in the F_2 generation by merely selecting the extreme cases of attack or non-attack. On the other hand, one cannot be so sure about the constitution of the moderately rusted individuals, for in this particular instance nearly one-quarter of these proved to be pure susceptibles. Most of the slightly rusted plants of 1918, however, turned out to be heterozygotes. Finally, a plant that bears traces of attack in one season may be shown by its offspring to be a genetically immune individual, while a rust-free plant may occasionally be proved a heterozygote which has escaped infection.

In considering the F_2 results it was seen that the 1 : 2 : 1 ratio was not very closely approached, though the figures strongly suggested its existence. The number of rust-free and badly rusted plants were, taken together, considerably less than those placed in the intermediate group. The probable explanation is that this deviation arises partly from the difficulty in determining the exact extent of attack by inspection only, and partly from the existence of other factors which are capable of modifying the degree of attack.

Table VIII. *Summary of results from 170 F_3 cultures in 1919.*

Extent of rust attack on the F_2 parents in 1918	Number of F_3 cultures raised, 1919	Condition of the F_3 cultures, 1919		
		All plants susceptible	Contained susceptible and resistant plants	All plants rust resistant
Very bad	24	24	—	—
Bad	19	16	3	—
Moderate	63	14	49	—
Slight	35	—	33	2
Traces	12	—	9	2
None	17	—	2	15
Totals	170	54	96	20

Table IX. *Approximate genetic constitution of the F_2 generation (autumn-sown portion) as regards susceptibility to Yellow Rust. These figures are arrived at by the direct application of the F_3 results.*

Number of F_2 plants placed in each grade for rust attack*	Results in F_3			Approximate genetic constitution of the F_2 crop as indicated by the F_3 results		
				DD	DR	RR
4 3 2 1 1r 0						
69		100% proved DD		69	—	—
17	{ 84 16	" DD " DR		98 —	— 49	— —
162	{ 22 78	" DD " DR		35 —	— 127	— —
210	{ 94 6	" DR " RR		— —	197 —	— 13
69	{ 75 25	" DR " RR		— —	52 —	— 17
202	{ 12 88	" DR " RR		— —	24 —	— 178
Totals:				202	419	208
The 1 : 2 : 1 ratio would be:				(207)	: (414)	: (207)

* For rust grades, see p. 61.

78 *Mendelian Inheritance and Yellow Rust in Wheat*

If, however, the results obtained from the F_3 cultures are applied to the number of plants placed in the different grades of attack in the F_2 , we find that the 1 : 2 : 1 ratio is very clearly demonstrated. The details of this application are set out in Tables IX and X, and show that the probable composition of the autumn-sown portion was approximately:

$$202\ DD : 419\ DR : 268\ RR,$$

and of the spring-sown portion of the F_2 generation:

$$189.7\ DD : 378.2\ DR : 163.1\ RR.$$

Table X. *Approximate genetic constitution of the F_2 generation (spring-sown portion) as regards susceptibility to Yellow Rust. These figures are arrived at by the direct application of the F_3 results.*

Number of F_2 plants placed in each grade for rust attack*						Approximate genetic constitution of the F_2 crop as indicated by the F_3 results		
4	3	2	1	1r	0	DD	DR	RR
86						100% proved DD	86	
	89					(84 .. DD	71.7	—
						(16 .. DR	—	14.3
		132				(22 .. DD	29	—
						(78 .. DR	—	163
			181			(94 .. DR	170	—
						(6 .. RR	—	11
				98		(75 .. DR	73.5	—
						(25 .. RR	—	26.5
					145	(12 .. DR	17.4	—
						(88 .. RR	—	127.6
Totals:						189.7	378.2	163.1
The 1 : 2 : 1 ratio would be:						(183)	(365)	(183)

* For rust grades see p. 61.

The total number of plants was 1560, and the numbers expected according to the 1 : 2 : 1 ratio are (390) : (780) : (390). Applying the F_3 results to the whole F_2 crop, we find the following composition indicated:

391.7 homozygous susceptible individuals,

797.2 heterozygous susceptible individuals,

371.1 homozygous immune individuals.

6. RESULTS OBTAINED FROM F_2 , F_3 AND F_4 CULTURES, ETC., IN 1920.

The foregoing results were supplemented by further data obtained during 1920. All the rust-cultures of that year were grown on the Uni-

versity Seed Farm near Cambridge. The ground, which had received a dressing of 10-12 loads of farmyard manure and had been cropped with potatoes in the previous year, was in good condition for wheat growing. Cultures of the F_2 , F_3 and F_4 generations of the cross *Wilhelmina* \times *American Club* were raised, in addition to others.

During the winter 1919-1920 abnormally mild weather was almost continuous. One interesting feature associated with this mild weather was that freshly formed uredospores of Yellow Rust were found from the first week in October, 1919, onward to the following summer on certain susceptible wheats which had been sown at the end of August, 1919. During the spring and summer of 1920 the weather was favourable to the normal growth of the cultures (see Table I).

Yellow Rust attack was general amongst the field cultures at a very early date, and by the beginning of May in many of the susceptible cultures all the plants were rusted. By the first week in June the attack was at its height.

(a) *Further F_2 statistics obtained in 1920.*

In raising the F_2 generation (cross No. 120) in 1918, no attempt was made to keep separate the grain of the several F_1 plants. In a later cross, however (*Brooker's* \times *American Club*, cross No. 154), this was done, and several distinct F_2 families were raised in 1920. Two of these families were examined in the same manner as the F_2 of cross No. 120 had been in 1918 with the following results:

A population of 198 F_2 individuals raised from a moderately rusted F_1 plant contained 42 badly rusted plants and 40 which remained absolutely rust-free. On the remaining plants, the rust varied from the merest trace up to a moderate attack.

The second F_2 family consisted of 258 individuals and was raised from an F_1 plant which had a slight attack only. It contained 62 badly rusted and 61 perfectly rust-free plants, the remaining 135 individuals being attacked to not more than a moderate extent in any case.

These figures indicate that similar results are obtained whether the analysis be made upon the progeny of separate F_1 plants or upon the offspring of several F_1 's combined.

The small F_2 crop of the original *Wilhelmina* \times *American Club* cross grown in 1920 contained 114 plants, and by the end of July it was found that 86 of these were rusted and 28 were rust-free. Of the rusted plants six had a much severer attack than *Wilhelmina* grown under similar conditions.

80 *Mendelian Inheritance and Yellow Rust in Wheat*

(b) Direct analysis of an F_2 through the F_3 results, 1920.

A small F_2 crop of the same parentage (Wilhelmina \times American Club) consisting of 86 plants was raised in 1919. No attempt was made to grade these for rust attack as it was decided to grow an F_3 culture from each plant and to use the F_3 data for placing each F_2 plant in its proper category for susceptibility or resistance. These 86 F_3 's were raised in 1920. In 22 of these cultures all the plants were readily susceptible; 20 others contained only rust-free or highly resistant plants, while the remaining 14 cultures consisted of readily susceptible and resistant plants. The inference is that the F_2 grown in 1919 consisted of 20 rust-resistant plants, 14 impure susceptible and 22 pure susceptible individuals. In four of the pure susceptible F_3 cultures all the plants were excessively attacked.

(c) Results from F_4 cultures.

(Cross No. 120, Wilhelmina \times American Club.)

A considerable number of F_4 cultures were grown in 1920. Twenty-seven of these were raised from plants taken at random out of the wholly susceptible F_3 cultures of the previous year. Without exception these F_4 plants (133 altogether) were badly rusted.

Thirty-six F_4 cultures were grown from plants which had been extracted from obviously segregating F_3 cultures. Space will not allow a full account of the results, but they showed definitely that the three genetic types—pure resistant, pure susceptible and impure susceptible—were present in the F_3 cultures. At the same time they indicated again that some allowance must be made for fluctuations in susceptibility due to season and other external conditions. The 19 F_4 's raised from rust-free F_3 plants consisted entirely of rust-resistant individuals. Similarly all the extracted badly rusted F_3 plants produced badly rusted F_4 cultures, while slightly or moderately rusted F_3 plants generally produced cultures in which rusted and rust-free plants occurred.

Two hundred and eighty-eight F_4 cultures were raised from F_3 plants which were the offspring of rust-free F_2 individuals.

Some of these were picked from cultures like 66/9, 11/21, 2/10 and 19/12 (Table III) in which all the plants were completely or almost completely rust-free in 1919.

The F_4 plants numbered several thousands and it was impossible to examine each individual very closely, but a general inspection showed that all the plants were either rust-free or possessed a very high degree of resistance.

Many plants were purposely taken from cultures 15/5, 21/3, 29/15, 57/8, etc. (Table III), in which rust-resistance had apparently been disturbed or "broken down" under the adverse conditions prevailing in 1919. A few of these extracted F_3 's had, indeed, been badly rusted, e.g. 15/5/15, 21/3/18, etc. Nevertheless in 1920 all the F_4 plants proved to be either completely rust-free or highly resistant.

Table XI. *Progress of infection by Yellow Rust on the various F_3 cultures in 1919. (Cross No. 120.) 169 cultures*.*

F_3 cultures 1919	Number of F_3 cultures	Percentage of cultures on which infection had occurred for the weekly periods ending									Final examination July 28th to August 11th
		May			June				July		
		17th	24th	31st	7th	14th	21st	28th	5th	12th	
<i>DD</i> 's	53*	13.2	34.0	77.3	94.3	100	—	—	—	—	—
<i>DR</i> 's	96	3.1	15.6	22.4	49.0	61.4	64.5	80.2	81.2	93.7	100
<i>RR</i> 's	20	0	0	0	0	0	0	0	0	0†	90

* One culture omitted because no record of date of first attack was made.

† Only one small pustule seen on one plant by this date out of a total of 817 plants.

SECTION III. SOME OF THE FACTORS WHICH MAY INCREASE OR DIMINISH SUSCEPTIBILITY TO RUST ATTACK.

INTRODUCTION.

In the previous section of this paper it was seen that a sharply defined 1 : 2 : 1 ratio was not directly found in the F_2 generation in 1918, although the F_3 results showed that it undoubtedly existed. The variations observed in the F_2 , and from season to season, appear to indicate that, in addition to the inherited factors which primarily determine resistance, etc., there exist other factors which may tend either to increase or reduce a plant's predisposition to attack.

The now generally accepted view is, that immunity is due to the production of specific toxins or anti-toxins which have the power to neutralize the action of the attacking fungus. If this is correct, an immune plant is one whose normal metabolism provides such a substance, whilst a susceptible plant is one which is either unable to form such a substance at all, or can only do so to an ineffective extent. But even in the case of a plant which is able to produce such protective substances, it is obvious that such production may be subject to modification as regards quantity, rate of formation, etc. There is, indeed, evidence to show that a state of complete immunity depends not only upon the inherited factor for resistance, but also upon a properly balanced con-

82 *Mendelian Inheritance and Yellow Rust in Wheat*

dition of the plant's normal metabolism. For example, American Club, though normally immune, has been found to develop pustules of Yellow Rust under abnormal conditions of growth(10) or nutrition(12). It is therefore probable that any factor or factors which bring about a condition of growth unfavourable to the production of those substances upon which immunity depends, may lead to a greater or less degree of susceptibility. Ward(20) especially emphasized the fact that "the physiological condition of the host is always a factor of prime importance" in considering the behaviour of the host towards the parasite.

Since all physiological processes are dependent upon both internal (inherited) and external factors, in matters of this kind we must be careful to distinguish between the inherited and non-inherited factors concerned in bringing about the net result. Consequently, in trying to discover the causes of the "disturbance in rust resistance" as observed for example in 1919, we must recognize the possible effects of:

- (1) External environmental factors.
- (2) Inheritable factors (other than those primarily concerned in causing resistance, etc.) which lead to fresh combinations of parental features in the hybrid and its descendants. These will now be considered.

(a) EFFECT OF CLIMATIC CONDITIONS.

The greater severity of attack in 1919 is partly explained by the different weather in the two seasons. That of 1918 favoured a regular and normal development of the plants. On the other hand the cultures of 1919, while still in a backward condition, were subjected first to a 7 weeks' drought, and afterwards to 6 weeks of almost continuous dull, cool, wet weather.

During both years records were taken of the extent of the Yellow Rust attack on 13 distinct varieties of wheat grown in the cages. In eight cases the attack was more severe in 1919 than in 1918; on four varieties it was of about the same intensity, and in one case it was slightly less severe. This and other evidence support the belief that the abnormal weather of 1919 was partly responsible for the more pronounced attack in that year.

(b) FOOD SUPPLY.

It was, however, clear that the greater intensity of attack on the F_3 cultures in 1919 was not due solely—or even chiefly—to the different weather conditions. The increased severity of attack was far more pronounced on these than on the standard varieties growing alongside.

while the only known difference in environmental conditions was that of food supply. The rust-cultures had all received a heavy dressing of nitrate of soda, whereas the other plots were unmanured. The nitrate was carried to the roots of the plants when their very existence was in the balance at the end of the drought (June 20th). Thus the necessary supply of moisture, and also a large quantity of available nitrogen, were simultaneously presented to the plants. It is very important to note, therefore, that the change from a critical condition to a state of active growth was as sudden as it was possible to be. The "second growth"—which also occurred on the unmanured wheats—was stimulated to an enormous extent on the F_3 's, and the cultures assumed the very dark green colour characteristic of plants receiving an excess of nitrogen. The maturation of the plants was also considerably delayed. These conditions evidently in some way afforded a greater opportunity for rust attack, for it was precisely during this period of delayed maturation that the great epidemic of the season developed.

Nitrogenous manures, especially when greatly in excess of other fertilizers, are well known to be very effective in increasing the severity of rust attack. Biffen(3) has pointed out that, on the Rothamsted wheat plots, rust attack is invariably encouraged where ammonium salts or nitrates are continuously applied in heavy doses. Spinks(12) also showed that susceptibility to Yellow Rust is increased by the use of large quantities of available nitrogen, while plants which are semi-starved as regards nitrogen may exhibit a considerable degree of resistance. Further, as Spinks and others have shown, some salts, e.g. salts of potassium and especially lithium salts, may markedly reduce susceptibility. The question of food supply is therefore certainly of great importance in connection with the observed fluctuations in susceptibility.

In connection with this question of food supply, it may be noted that during the present experiments there appeared to be a difference in susceptibility between "interior" and "exterior" plants in the same pure susceptible cultures. Records of the first plants to be attacked were made on 23 of the homozygous susceptible cultures given in Table VII. At the time of "first infection" these cultures contained 642 "interior," and 124 "exterior" plants. By June 7th the number of infections noted on "interior" plants was 35, i.e. 5.4 per cent., while at the same date the infected "exterior" plants numbered 20, i.e. 16.1 per cent. Similarly, in 12 other pure susceptible cultures, by the end of May it was found that 7.3 per cent. of the "exterior" plants were attacked, but only 2.3 per cent. of the "interior" plants. The number of early infections were

84 *Mendelian Inheritance and Yellow Rust in Wheat*

therefore relatively three times as numerous on the "exterior" as on the "interior" plants.

An experiment was planned in order to find out if possible to what relative extent such circumstances as wide spacing and large amounts of available nitrogen were responsible for the increased severity of attack observed. The varieties included in the test were the two parent wheats *Wilhelmina* and *American Club* and the following extracted F_3 types descended from these parents:

66/9/*d*, a rust-resistant type, with white chaff and beardless medium-lax ears (a resistant *Wilhelmina* type).

82/11/*a*, a rust-resistant type, with red chaff and beardless medium-lax ears.

75/7/*c*, a susceptible type, with white chaff, and dense bearded ears (a white chaffed susceptible *American Club* type).

75/11/*d*, a dense eared, bearded, susceptible type with red chaff. (This was practically a susceptible *American Club*.)

Four beds, *A*, *B*, *C*, and *D*, each 1 foot wide, were laid out, and each variety was sown across these in a single row 16 feet long. A space of 6 inches was allowed between each row, but the grains were sown 2 inches apart on beds *A* and *D*, and 12 inches apart on *B* and *C*. The grain was sown on November 24th, 1919, and a fairly uniform plant was obtained.

On May 4th, 1920, beds *A* and *B* were top-dressed with nitrate of soda at the rate of 7 cwt. per acre. It should be noted that, although beds *C* and *D* received no nitrate, the soil was in good condition after the potato crop of the previous year; the comparison to be made was not between starved and overfed plants, but between plants grown under normal and abnormal conditions as regards space and nitrogenous manuring.

Yellow Rust was first seen on May 7th on one or two plants of 75/11/*d* and *Wilhelmina* (exterior row) in each case on bed *A*. By May 12th rust attack had become general on all the susceptible varieties, the only difference being that it was of a less pronounced character on *Wilhelmina*. At that date no sign of attack could be found on any of the resistant varieties although their foliage was literally powdered daily with uredospores from their susceptible neighbours.

The condition of the plants on May 21st and June 2nd is shown in Table XII in which the numerator of each fraction indicates the number of plants rusted out of the total surviving plants of each variety on each bed (given as the denominator).

General condition of the plants on May 21st, 1920.

The plants on bed *A* were of a dark green colour and growing vigorously; they were about 2 feet high, and owing to the close planting and strong growth the shoots were densely crowded together. Mildew was abundant, especially on Wilhelmina and 66/9/*d*.

Table XII. *Effects of wide planting and heavy application of Nitrate upon wheats susceptible and resistant to Yellow Rust. Grain sown Nov. 24th, 1919. Nitrate of soda applied May 4th, 1920, at the rate of 7 cwt. per acre.*

Proportion of plants attacked by June 2nd, 1920		Proportion of plants rusted by May 21st, 1920																					
A	B	C	D	No. of row	Variety of wheat	1	2	3	4	5	6	7	8	9	10	11	12	Nitrate applied					
						Wilhelmina (exterior row) [moderately susceptible]	American Club	Burbank's [super-susceptible]	66/9/d [an extracted resistant F ₄]	75/7/c [an extracted susceptible F ₄]	American Club	Burbank's	82/14/a [an extracted resistant F ₄]	75/11/d [an extracted susceptible F ₄]	American Club	Wilhelmina [interior row]	Wilhelmina [exterior row]						
Every plant rusted	Every plant badly rusted	Every plant badly rusted	Every plant badly rusted	15	0	13	0	14	0	19	0	14	0	15	0	16	20	Nitrate applied					
						18	18	14	19	19	22	14	19	17	15	20	22						
						3	0	4	0	3	0	4	0	3	0	4	0		4	0	No nitrate applied		
						3	4	4	3	3	2	4	2	3	4	4	1						
						12	0	3	0	5	0	12	0	4	0	12	3		No nitrate applied				
						4	2	3	5	5	3	12	2	4	2	12	3						
						2	0	10	0	7	0	17	0	11	0	10	16			No nitrate applied			
						15	15	14	18	13	18	17	19	19	13	12	17						
						3*		4		6		0		4								Nitrate applied	
						18		19		12		19		15									
						12		2*		1		12		0									No nitrate applied
						4		3		12		12		4									
0		0		1*		0		0				No nitrate applied											
12		5		3		12		2															
0		0		0		0		0					No nitrate applied										
15		18		18		19		13															
Every plant rusted																							
Every plant rusted																							
No nitrate applied																							
Nitrate applied																							

* In these cases the plants were only "flecked."

86 *Mendelian Inheritance and Yellow Rust in Wheat*

On beds *B* and *C* the plants were also making a very strong growth. Owing to the large amount of space available, numerous tillers were formed and the shoots were spreading out obliquely.

On bed *D* the plants were about 18 inches high, erect, and of a light green colour. They had tillered much less freely than the plants on the other beds.

Table XII shows that on this date all the plants of American Club and of the two extracted resistant types (altogether 205 plants) were still entirely free from infection. Taking the proportion of plants infected and the severity of attack into consideration, Burbank's was distinctly the most susceptible type, 68 out of 72 plants being badly attacked. The proportion of rusted plants in *Wilhelmina* and the two extracted susceptible types were very similar, but the attack on *Wilhelmina* was less severe; the actual numbers were: in 75/7/c, 34 rusted out of 40 plants; in 75/11/d, 33 rusted out of 43 plants; and in *Wilhelmina*, 91 rusted out of 121 plants.

Confining our attention for the moment to the susceptible varieties, we get some idea of the extent to which "wide spacing," etc., apparently favour rust attack.

The effect of "wide spacing" is seen by comparing beds *C* and *D*. On bed *D*, out of 107 plants of the susceptible varieties, 73 were rusted, i.e. 68 per cent. On bed *C*, 21 out of a total of 23 susceptible plants were rusted, i.e. 91 per cent.

The effect of the heavy dose of nitrate is seen from a comparison of beds *A* and *D*; on the former, out of 124 plants of the susceptible varieties, 111 were rusted, i.e. 89.5 per cent, as compared with 68 per cent. on *D*.

The combined effect of "spacing" and "nitrate" is seen by comparing beds *B* and *D*. On the former, 21 out of 22 susceptible plants were rusted, i.e. nearly 100 per cent. against the 68 per cent. on *D*.

It is clear from these and other observations that very widely spaced plants are more liable to an early infection than are closely planted individuals—other conditions being equal. A partial explanation for this possibly lies in the fact that widely spaced plants tiller more freely, and so offer a much greater area of leaf surface to infection than more crowded individuals. On the other hand the percentage of early infections on the closely planted bed *A* was as high as on the widely spaced bed *C*, and it is unlikely that this effect was brought about by the increased tillering of the plants on *A*. The increased susceptibility of plants receiving heavy doses of nitrogenous manures or extra space for growth appears to depend rather upon the increased or modified food-supply offered.

Condition of the plants on June 2nd, 1920.

By this date rust attack was at its height and the state of the beds was very interesting (Table XII).

It will be seen that every plant of Burbank's, Wilhelmina, and the two extracted susceptible types was rusted. On Wilhelmina the attack was generally speaking of a moderate character; on the other susceptible varieties it was very severe. The two extracted rust-resistant types and American Club continued to show very high resistance to attack, but in a few cases this resistance had apparently been more or less broken down. These cases will be noticed after first comparing the number of such apparent "breakdowns" on the different beds.

On bed *D* there were 83 plants of the immune varieties, and these all remained completely rust-free; not the slightest sign of infection could be found throughout the season.

On bed *C* only one plant was "flecked" out of a total of 14. Out of 15 plants on *B* 7 either bore pustules or were "flecked," while on bed *A* 17 plants were either "flecked" or bore pustules out of 93 individuals. The percentage of more or less successful attacks at this date on beds *D*, *C*, *A* and *B* was therefore respectively 0, 7, 18 and 40. These results point in the same direction as those given above for the number of early infections recorded among the susceptible varieties on the same beds.

These cases of infection were distributed among the "immune" varieties as follows: 17 cases occurred out of the 118 American Club plants; 6 occurred amongst the 45 plants of 66/9/*d*; while only 2 cases were met with out of 12 plants of 82/14/*a*.

The following particulars may be given of the individual plants concerned.

American Club. On bed *C* (row 6) 1 plant was slightly flecked. On bed *A* (row 2) 3 plants were clearly flecked though no pustules were formed. In row 6, out of 22 plants, 6 showed signs of infection. Plant No. 5 had a pronounced attack—though chiefly confined to one leaf. On this single blade, 71 well-developed pustules were counted, every one shedding spores, in addition to 17 other unbroken pustules. Plant 16, besides being flecked, bore three large pustules, one of which was shedding spores. Plant 19 had 7 well-developed pustules, 4 of which were broken, besides numerous flecks. Plants 6, 10 and 22 were flecked. In row 10, plant 2 was flecked and bore 47 pustules, many of which were broken. Plant 9 bore flecks and 2 unbroken pustules. Plants 12 and 15 were

also flecked, and the former had 8 pustules, some of which shed spores.

On bed *B* (row 2), 1 plant was slightly flecked, and another bore a few unbroken pustules on one blade. In row 10 the 1 plants remained free from infection. In row 6 there were only 2 plants; one of these had 14 leaves badly attacked and on these over 200 pustules were counted, many of which were freely shedding spores. Some weeks later it was discovered that this plant was also infected with Bunt (*Tilletia caries*)¹.

Turning next to the extracted resistant types it is seen that the plants of 66/9/*d* (row 1) on beds *C* and *D* were completely resistant on June 2nd; further, they remained immune to the end of the season. On bed *A*, 4 plants out of 19 had slight attacks, in each case a few pustules were formed, some of which were broken. On *B*, 2 out of the 3 plants had a few slight "flecks," but no pustules were seen. On the whole therefore this extracted F_4 type was distinctly more resistant than its resistant parent (American Club) under these abnormal conditions.

All the plants of the extracted resistant type 82/11/*a* (row 8) remained free from any trace of infection throughout on beds *A*, *C* and *D*. The 2 plants on bed *B* each had a slight attack on one blade. On one of these the attack was confined to the leaf-tip where the tissue was dying off. This extracted type was then decidedly more stable in its resistance than American Club.

In summing up, it may be repeated that the conditions under which these plants were grown were much more unfavourable to rust resistance than those likely to be met with in general farm practice. This is especially true of beds *A* and *B*. During the latter part of May and throughout June the susceptible varieties produced enormous quantities of uredospores and the alternating rows of resistant cultures were literally powdered all over day by day with fresh spores. It is conceivable that an occasional spore may possess more than the average power for bringing about an infection, and with such huge numbers of inoculations occurring day by day it is not surprising that now and then infection should have been accomplished.

But the evidence shows that the success of the parasite was of a very limited nature. Later observations indicated that the position on June 2nd was the worst reached during the year. Not only did the fungus

¹ Since then another similar case has been observed. In an F_3 culture (from the cross *Wilhelmina* × American Club) raised from an immune F_2 plant all except one plant were rust-free. This odd plant was very severely attacked by rust, and was also found to be infected with Bunt.

fail to infect any other plants, but even the infection areas already noted did not spread, and the tissues lying around such areas gradually died off and with them the parasite also. Numerous brown dead patches in the tissue remained, but clearly the hosts were the victors in the struggle.

The experiment shows that, while heavy doses of nitrate do lead to an intensification of rust attack on susceptible varieties, such treatment of normally immune wheats fails actually to break down or destroy their resistant powers. Under such treatment, however, the struggle which goes on between invader and host is seriously prolonged and may lead to an appreciable loss of leaf-tissue in the latter.

(c) NEW COMBINATIONS OF PARENTAL CHARACTERS.

It was clear, however, that, in addition to such external factors as those mentioned above, other causes were concerned in affecting the plants' susceptibility. In the same season (1919), and under the same external conditions, some of the homozygous susceptible cultures and plants were more severely attacked than others. Similarly, among the homozygous "immune" cultures (and plants) great differences existed in regard to the degree of resistance shown. Two of these cultures (66/9 and 11/21), containing 77 plants, actually came through this most unfavourable season, and in spite also of the heavy application of nitrate, *matured off absolutely free from Yellow Rust infection*. Two other cultures, including 91 plants, survived the test almost equally well, only 7 plants showing any sign of attack. These cultures did not escape by maturing off earlier than the rest, for they were not ripe until the second week in August. In the other pure "immune" cultures, however, while some plants were rust-free or had traces only, the majority had a slight attack, and a number were moderately rusted.

The above facts are only explicable on the assumption that the differences are partly due—at least indirectly—to other inherited features. It was observed that new combinations of such characters as ear shape, chaff colour, bearded or beardless condition, etc., appeared to be without effect upon a plant's susceptibility. But this does not involve the assumption that all combinations of other characters are likewise without effect. In a paper recently published Hayes⁽⁸⁾ points to some evidence of linkage between rust resistance and certain morphological characters in the offspring resulting from crosses between *Triticum vulgare* and varieties of *T. durum* and *T. dicoccum*. It is, indeed, extremely probable

that fresh combinations of those features which more especially affect the plant's metabolism may also modify its power of resistance. The parents of the cross under consideration were dissimilar in respect of several such characters, e.g. foliage, period of ripening, and probably also in root-range. As Biffen⁽⁵⁾ has pointed out, when segregation occurs fresh combinations of these various features result, and consequently the metabolism of such offspring is affected in various ways. The resulting root-range may be disproportionate to the new leaf-area, a slower rate of maturation prolongs the period of possible attack, and so on. Most important of all, it must not be overlooked that physiological features are also inherited, and re-combinations of these may produce unexpected results.

While some of these new combinations may bring about increased susceptibility, there was evidence that others may reduce it, or stabilise the inherited resistance. This fact has also been independently noted by Hayes⁽⁸⁾ in his experiments. These effects are realized not only in the F_2 , but also in any subsequent generation in which such combinations of features are inherited, or arise. A consideration of such points as these enables one to understand partly, at least, why statistics of rust attack on an F_2 generation (from a cross between susceptible and immune parents) seldom give a close approximation to the 1 : 2 : 1 Mendelian ratio.

(d) VARIATION OF SUSCEPTIBILITY IN HYBRID WHEATS.

The view that the re-combination of parental characters may lead to a modification of a plant's predisposition to attack is further supported by the various degrees of susceptibility possessed by hybrids. It has been stated by several observers that hybrid wheats are usually as susceptible to rust as the more susceptible parent. Instances, however, are known to the writer in which the attack was distinctly more severe on the hybrid, and also other cases in which it was much less severe.

In order to make a strict comparison on this point, certain hybrids were grown alongside their parents in 1919 under exactly the same conditions as to time of sowing, space allowance, etc. One of these crosses was between American Club and a very susceptible race No. 109/1. Of the 14 hybrids, none had more than a slight attack at the final examination on July 31st, while all the plants of 109/1 were badly rusted.

Another cross was made between a moderately susceptible variety (Brooker's) and American Club. Sixty-eight F_1 plants were raised in

1919. Rust varied from mere traces up to a mild attack on the hybrids whilst the susceptible parent had a moderately bad attack.

As far as these results go, they indicate that crosses between a normally immune variety like American Club and a susceptible variety give rise to hybrids whose inherited susceptibility is of an intermediate nature.

This, however, does not appear to hold good when some degree of susceptibility is possessed by both parents. In a further cross (No. 155) between Wilhelmina and Persian Black (a variety of *T. dicoccum*), the 33 F_1 plants raised were all very severely attacked. Both parents grown alongside under exactly the same conditions had only a moderate attack. Possibly the much increased severity of attack in this case may be due to the fact that there is a tendency for the hybrids of this cross to be sterile.

Two other cases may be mentioned: Rivet (*T. turgidum*—slightly susceptible) crossed with Persian Black (moderately susceptible), and Rivet crossed with Chinese White—a very early maturing susceptible variety—produced hybrids which were much more severely rusted than either of the parents.

In the three last-mentioned crosses none of the parent varieties are completely resistant to attack, and as the other differences between them are unusually great and numerous, it is possible that the increased susceptibility of the hybrids may be largely due to the altered metabolism of the plants.

(e) POSSIBLE EFFECT OF ENVIRONMENT UPON THE FUNGUS.

In conducting these investigations, it has not been forgotten that the cause of the disease is also a living organism favoured, or disfavoured, by factors similar to those that affect the host, and that it must therefore also receive consideration in dealing with the question of variation in susceptibility of the host.

Undoubtedly weather conditions affect the general spread of the fungus according as they favour the vitality, dissemination, and germination of its spores, or otherwise. But apart from this, the writer has had no evidence in the field that the parasite is *directly* aided in its attacks by one kind of weather rather than another. The spread of the disease in susceptible plants was as rapid during the hot, dry period from May 13th to June 19th in 1919 as it was in the cool, damp period from June 20th to July 31st of the same year.

92 *Mendelian Inheritance and Yellow Rust in Wheat*

It has been supposed that increased virulence is gained by a rust after passing one stage of its life-history on a congenial host. For example, Pole-Evans(6), working with *P. graminis*, believed that his experiments showed (a) "that the F_1 from a cross between a susceptible and an immune wheat is more susceptible to rust attack than its susceptible parent," and (b) "that the rust which passes through a hybrid plant produces a far more severe infection than the rust from the susceptible parent." Neither of these conclusions, however, appear to carry much weight since the wheat employed as the "immune" parent proved on some occasions to be moderately susceptible. As the present investigations have shown, hybrids which result from the crossing together of two more or less susceptible varieties are generally more susceptible than either of their parents. The second conclusion referred to has recently been negated by the experiments of Stakman(13) who worked with the same rust. Stakman's evidence showed that the pathogenicity of rusts is not easily changed by host-influence. He says "although many attempts were made to increase the virulence of biologic forms on resistant hosts, the results indicated that rust-forms do not gradually adapt themselves to resistant or semi-congenial hosts."

Since rust spores probably vary as regards their capacity for infection, an occasional spore may be able to bring about a more or less successful infection on a host plant which is capable of complete resistance in the vast majority of cases. In this way perhaps the partial or temporary breakdown of rust-resistance may be partly accounted for. At present, however, the bulk of the experimental evidence indicates that it is to the physiological condition of the host, rather than to any increased virulence on the part of the parasite, that variations in a plant's susceptibility are due. Changes in the metabolism of the possible host are brought about in various degrees by the interaction of both inherited and non-inherited factors, and it is suggested that such changes may lead to a diminished resistance by proving unfavourable to the formation of those products upon which immunity depends.

CONCLUSIONS.

The evidence obtained from the investigations described demonstrates:

1. That immunity and susceptibility to Yellow Rust attack are inheritable features in wheat.
2. That susceptibility and immunity behave as unit-characters, and

depend primarily upon definite factors which are inherited according to the simple Mendelian law.

3. That segregation leads to the occurrence in the F_2 generation of susceptible and immune individuals in the proportion of 3 : 1.

4. That the immune F_2 plants breed true to that character.

5. That one-third of the susceptible F_2 plants are homozygous for susceptibility and breed true, and that the remaining two-thirds are heterozygous for susceptibility and give rise to offspring in which rusted and rust-resistant plants occur as in the F_2 .

6. That homozygous susceptible plants are distinguishable from heterozygotes quite as much by an earlier and more rapid spread of infection as by the final extent of attack.

7. That a plant's predisposition or resistance to attack is, nevertheless, subject to greater or less modification from the interaction of various other causes. There is evidence for this in the fact that a badly rusted plant may prove to be heterozygous for susceptibility, whilst a moderately rusted individual may be shown to be homozygous for the same feature.

8. That the relative difference in resistance between homozygous susceptible and "immune" plants remains approximately the same even when the external conditions are favourable to an extremely severe attack.

9. That while new combinations of characters may lead indirectly to increased susceptibility, there is evidence to show that the reverse effect may be produced. In some instances the inherited immunity was in some way so stabilised that the most favourable conditions for rust attack failed to bring about the slightest degree of infection.

10. That breeding for rust resistance may proceed with every assurance of success, and that even the production of new races as stable for immunity as American Club itself is by no means an impossible task.

The evidence further indicates:

11. That a cross between a normally immune and a susceptible wheat produces a hybrid in which the inherited susceptibility is probably of an intermediate nature.

12. That the causes referred to above under clause 7 probably include any factor which is capable of modifying the plant's metabolism to an appreciable extent, and embrace

(a) Inherited factors leading to fresh combinations of morphological or physiological features, and

(b) Non-inherited environmental factors.

APPENDIX.

*An attempt to estimate the Reduction of Yield due to
Yellow Rust attack.*

Various estimates have been given as to the probable loss of grain due to rust attack, but most of these appear to be little more than guesses. It is, of course, certain that rust attack may be so severe as to destroy the host plants altogether, but such super-susceptible races do not enter into general cultivation in countries where they are liable to attack. The question to be answered is, What is the approximate reduction in yield of those varieties which, though capable of being generally cultivated are nevertheless subject to a moderate or occasionally a bad attack?

A favourable opportunity occurred to test this point in 1920.

A variety of wheat known as "Jap" has been grown on small plots at Cambridge for the last 15 years. It was formerly used as a parent, but in recent years has been discarded for this purpose owing partly to the inferior nature of its straw and partly because of its susceptibility to Yellow Rust. A small culture has, however, been grown on each year, and in 1918, when the parent stocks were examined as to their comparative susceptibility, it was noticed that the "Jap" culture consisted of plants which varied widely in this respect. Looking at the plot as a whole, it appeared to be suffering from a moderate rust attack, but when examined in detail, certain plants were seen to be rust-free. A final inspection of the culture in July showed that it contained 38 rusted and 14 rust-free individuals. Six of the rusted and four of the rust-free plants were saved, and from these separate cultures were grown in the following year. Owing to the extremely favourable conditions for rust attack in 1919 the cultures raised from the rusted plants were attacked with great severity, and even those grown from the rust-free plants had a slight attack. The relative difference in their power of resistance appeared to be the same as in 1918. All the cultures were of the "Jap" type as regards straw, ear-shape, etc., and if morphological differences existed they were too slight to be apparent to the naked eye. The only observed difference apart from rust resistance was in the period of "ear-emergence," there being some 10 days between the earliest and latest in this respect. Rust susceptible and resistant cultures were included in both the early and late groups.

One plant (3/16/29) was saved from a pure susceptible culture (3/16), and also one plant (1/4/10) from a resistant culture (1/4), and a plot of each was raised in 1920; a similar plot was grown from the original

mixed stock of "Jap." On May 12th, 1920, out of 57 plants in culture 3/16/29, 41 were attacked by Yellow Rust; on the same date the 66 plants in culture 1/4/10 were free from infection. Culture 3/16/29 was about 7 days earlier than the other, and all the plants in it were rusted by June. Only a few slight traces of attack and some "flecked" plants were found on culture 1/4/10 before harvesting.

The original intention in growing these cultures was merely to see whether the extracted types bred true to resistance and susceptibility respectively, but, at harvest-time, they seemed to offer very favourable material on which to determine the loss directly due to Yellow Rust. In the first place, their identical—or almost identical—morphological features appeared to afford safe ground as a basis for such a comparison. Again each culture had remained free from attack by Brown Rust or other fungi up to the time the plants were pulled—July 23rd—so that the difference in yield was chiefly, if not entirely, due to the presence or absence of Yellow Rust. Further, each plot was sown on the same day, grown under identical conditions side by side, and no loss of grain had occurred at harvest-time. In one respect only there was a slight difference between the cultures, viz. in the number of plants per row, this being due to the rather poorer germination of the grain in the susceptible cultures. The results were as follows:

				Culture of "Original Mixed Jap"	Culture 1/4/10 "Resistant"	Culture 3/16/29 "All susceptible"
Rows	3	4	4
Plants	45	66	57
Plants per row	15	16.5	14.2
Total weight of ears (grams)				310	555	245
Weight per plant (grams) ...				6.9	8.4	4.3
<i>Percentage relative weights:</i>						
(a) Per plant	82.1	100	51.2
(b) Per unit area	74.1	100	44

It will be seen that the weight of ears in the "mixed" culture was reduced by 18 per cent. per plant, and by nearly 50 per cent. per plant on culture 3/16/29. The reduction per unit area was still greater in each case. Had the grain been threshed out and weighed separately, it is certain that the differences would have been still more striking, but this unfortunately was not done.

These figures indicate that a variety of wheat having on the average a moderate degree of susceptibility (comparable with, say, Square Head's Master) may give a yield at least 25 per cent. below that obtainable from almost precisely the same form when rendered rust-resistant.

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NOTE ON THE COMPOSITION OF A FLUID OBTAINED FROM THE UDDERS OF VIRGIN HEIFERS

BY HERBERT ERNEST WOODMAN, PH.D., D.Sc.,
AND JOHN HAMMOND, M.A.

*(From the Institute for the Study of Animal Nutrition,
School of Agriculture, Cambridge University.)*

THE secretion of milk by the mammary glands normally follows a period of pregnancy, but numerous cases have been cited in the literature of secretion which takes place in animals that have never borne young. Non-pregnant bitches frequently secrete milk several weeks after oestrus(1), and a secretion, apparently similar to milk, takes place after pseudo-pregnancy in rabbits(2). No quantitative analyses have been made of these secretions to show whether they possess the characteristics of true milk or colostrum.

The origin and significance of colostrum itself, as distinct from milk, is also in doubt, and its formation has variously been attributed to: (1) The break up of the central cells of the alveolus, or as a result of their initial activities; (2) The filtration of lymphatic secretion through the walls of the alveoli mixing with the milk formed by the cells. Recent work on the proteins of colostrum(3) has shown that although the caseinogen and albumin must be elaborated by the mammary gland, yet the globulin, which is present in large amount in the colostrum secretion, is in every respect identical with the globulin of blood serum.

There also exists the possibility that colostrum results from the partial absorption of the more diffusible milk constituents, the secretion of which has been taking place to a small extent some time previously.

It was therefore of considerable interest to find, during a study which is being made by one of us (J. H.) of the development of the udder of the cow, that the galactophorous sinuses and ducts of virgin heifers of some $1\frac{1}{2}$ to $2\frac{1}{2}$ years of age contained very frequently a fluid which could often be expressed from the nipples in quite considerable amounts. The object of the present work was to investigate the composition of the secretion and its possible relation to colostrum and to milk.

It has been suggested by several writers(4) that the mammary gland

undergoes a cycle correlated with that existing in the ovaries and culminating at oestrus by a swelling of the mammary gland. Since heifers have periodic corpora lutea and a well-marked ovarian cycle, it might be supposed that the former acted in much the same way as those of pseudo-pregnant rabbits, but to a smaller extent. It was not unreasonable therefore to suppose that this fluid was secreted just before the period of oestrus in heifers, but attempts to prove this so far have been without success. The fluid can be drawn off from the udder at any period of the cycle, and the amount varies considerably according to the individual. In animals killed at various periods of the oestrous cycle, there was an indication that more existed just before the oestrous period than at any other time. But individual cases were also found which did not show this behaviour.

The secretion of milk has been attributed to the removal of the stimulus which caused the mammary gland to develop. Thus Lane-Claypon and Starling⁽⁵⁾ and others have attributed this action to the foetus, but Marshall and Hammond⁽²⁾ found that the corpus luteum caused the growth of the mammary gland in the rabbit, and that milk was produced whenever the gland had developed to a sufficient extent.

The removal of the causative stimulus in heifers cannot, however, be the cause of the secretion, as the fluid has been found in the udders of previously virgin heifers pregnant 3-4 months, at a time when the corpus luteum is still large and active.

The problem thus presented itself: Is the secretion similar to that of colostrum and milk, resulting from the activity of the cells of the alveoli, or is it merely an exudation of lymph filling up the galactophorous sinuses, which have been formed by the development of the mammary gland? If the latter hypothesis be found correct, then at what stage in the development of the mammary gland do the typical constituents of milk first appear?

CHEMICAL INVESTIGATION OF THE FLUID.

In all, four samples of the fluid were examined. The first two samples were obtained from heifers which had never been served, whilst the third and fourth samples were taken from the udders of the heifers during the first three weeks of pregnancy.

The amount of fluid was only small, each heifer contributing on an average about 7 c.c. to the supply. The liquid was slightly opaque in appearance, though not possessing the dense opaqueness of milk. A slight sediment settled out on standing, but the fluid passed fairly

readily through a filter paper, the filtrate then being almost clear and possessing the appearance of a protein solution. It was slightly viscous, gave a foam on shaking and did not show the amphoteric reaction of fresh milk, but was very faintly alkaline to both litmus and phenolphthalein.

A preliminary investigation of the first two samples was carried out as follows. A small volume of the fluid was diluted with distilled water and one drop of acetic acid was added. On shaking, a white flocculent precipitate settled out in a manner characteristic of the separation of caseinogen from milk by this method. This was filtered off and was shown to be a protein by the usual tests. Furthermore, it answered to all the characteristic tests for caseinogen. (1) It was insoluble in water but dissolved readily in dilute soda and in lime-water and was reprecipitated by the addition of a drop of acetic acid. (2) When fused with a mixture of K_2CO_3 and KNO_3 , the solution obtained on extracting the residue with water gave a strong phosphate test with nitric acid and ammonium molybdate. (3) The freshly precipitated protein was completely soluble in excess of acetic acid.

Further tests were made in order to ensure that the substance was not a nucleoprotein, which class of bodies gives most of the general reactions of the phosphoproteins. The presence of nucleoproteins in such a fluid would, moreover, not be at all surprising, especially if it resulted from the filtration of lymph through the walls of the alveoli. That the protein was caseinogen, however, and not a nucleoprotein was shown by: (1) The readiness with which it split off its phosphorus as inorganic phosphate by mild alkaline hydrolysis. Under these conditions, nucleoproteins do not yield phosphoric acid, the phosphorus remaining bound up in the nucleic acid group(6). (2) The behaviour of the fluid towards rennet. To 5 c.c. of the fluid were added 2 c.c. rennet extract. On placing in a bath at 40° , only a slight turbidity resulted after two minutes. On the further addition of two drops of calcium chloride solution to the mixture, however, the turbidity increased and a curdy precipitate settled out. A clear mixture of 5 c.c. fluid, 2 c.c. rennet extract and 1 c.c. calcium chloride solution was placed in a bath at 40° . In a few seconds, a turbidity spread throughout the liquid, followed by a rapid separation of curdy precipitate. This behaviour was consistent with the presence of caseinogen in the fluid, the rennet producing, in the presence of calcium salts, a precipitate of insoluble calcium caseate from the soluble calcium caseinogate. (3) The absence of any insoluble nuclein residue when the protein was submitted to peptic digestion.

The filtrate remaining after separation of the caseinogen was heated a short time in boiling water. This caused coagulation and the substance which separated in this manner was shown to be protein by the ordinary tests. The nature of the coagulable proteins was investigated in the following manner. After separating the caseinogen from another portion of the fluid, the clear filtrate was made exactly neutral by means of $N/10$ NaOH and was then saturated with magnesium sulphate. The protein which was salted out by this process was filtered off and re-dissolved in water. On saturating this solution with magnesium sulphate, the protein was again thrown out. It possessed therefore the characteristics of a globulin.

The filtrate from the globulin was next acidified with acetic acid. A further precipitate of protein was obtained, its mode of isolation proving it to be an albumin. As far as it was possible to judge from the tests, the fluid contained rather more globulin than albumin.

The liquid remaining after removal of caseinogen by acetic acid and albumin and globulin by coagulation still answered to the biuret test and gave an appreciable precipitate with tannic acid. It still contained nitrogenous bodies of a simpler type, amongst which was a proteose, since a further precipitate was obtained on saturating the liquid with ammonium sulphate. The existence of non-protein nitrogen in the fluid was borne out by subsequent analysis.

The fluid, after removal of proteins, was found to exert a slight reducing action when boiled for some time with Fehling's solution. The osazone, which was only obtained in small amount and with difficulty, had the characteristic appearance of lactosazone under the microscope. A sample of milk, tested similarly, showed ready and copious reduction of Fehling's solution and gave a good yield of lactosazone without difficulty. In all the samples of fluid examined, it was only possible to demonstrate the presence of lactose in small amounts.

A preliminary extraction of the fluid with ether showed it to contain a small amount of fat. Sample 4 was found, by the Paper Coil method, to contain about 0.6 per cent. of a solid yellow fat. This was saponified by boiling a short time with caustic soda. After acidifying, extracting with ether and allowing the solvent to evaporate, the residue possessed the characteristic odour of butyric acid. It followed that the fat in the fluid was the product of mammary synthesis.

The fluid did not contain any detectable amount of mucin, since this would have shown as gelatinous strands on the addition of acetic acid. The precipitate thus obtained was, however, perfectly flocculent

and was, moreover, completely dissolved by the addition of excess of acetic acid. Mucin remains undissolved under these conditions. The perfectly flocculent character of the precipitate obtained on adding alcohol to the fluid and stirring precluded the possibility of the presence of pseudo-mucins in the fluid. No other glucoproteins were found in the fluid, since on hydrolysing the total protein with hydrochloric acid, no reducing sugars were formed.

The main constituents of the fluid were, therefore, caseinogen, globulin and albumin, with traces of fat, lactose and simpler nitrogenous substances (proteoses). In view of the obvious importance of the fluid in its relationship to the ultimate colostrum and milk secretions, it was advisable, as far as was possible, to conduct a quantitative enquiry. Unfortunately, no one sample obtained was large enough to permit of a complete analysis being carried out on it. The following figures were, however, obtained and give some idea of the quantitative composition of the fluid. The data for milk and a sample of colostrum containing roughly the same amount of protein are appended for comparison.

Samples 1 and 2.

Total protein (total N \times 6.37)	5.82 %
Caseinogen	1.70 "
Coagulable proteins	3.29 "

Sample 3.

	Fluid	Colostrum ¹	Milk ²
Total protein (N \times 6.37)	6.37 %	6.18 %	—
Caseinogen	2.50 "	3.25 "	2.90 %
Globulin ³	2.28 "	1.97 "	Trace
Albumin	1.00 "	0.70 "	0.60 "

Sample 4.

	Fluid	Milk ⁴	Colostrum ⁵
Specific gravity	1.014	1.032	1.050
Total solids	8.80 %	12.25 %	14.19 %
Ash ⁶	0.80 "	0.75 "	0.96 "
Fat ⁷	0.63 "	3.40 "	4.21 "

¹ Crowther and Raistrick, *Bioch. J.*, **10**, 435, 1916. The sample represented the fourth milking after parturition.

² Fleischmann.

³ The globulin was separated from the liquid, remaining after removal of caseinogen with acetic acid, by neutralising with N/10 NaOH and saturating with magnesium sulphate. After filtering, the precipitate was well washed with saturated magnesium sulphate solution and its amount estimated by the Kjeldahl method. The albumin was isolated from the filtrate by acidifying with acetic acid and standing in boiling water for a short time.

⁴ Fleischmann.

⁵ Eugling. Sample taken 48 hours from parturition and contained about 6 % protein.

⁶ The ash gave the reactions for phosphate and calcium.

⁷ Paper Coil method.

CONCLUSIONS.

A secretion has been found to occur in small amount in the udders of virgin heifers which contains the characteristic proteins of colostrum, together with slight amounts of fat, lactose and proteose. It follows that the initiation of mammary gland activity in the dairy cow is not necessarily dependent on pregnancy, but may be associated with the occurrence of the oestrous cycle. The processes which result in the elaboration of proteins would appear to be the most active in this early stage of the gland's activity, since only small amounts of fat and sugar seem to be formed.

The fluid bears, on account of its globulin content, a closer relation to colostrum than to milk. The question arises as to whether the secretion continues to accumulate throughout pregnancy, or whether it undergoes gradual reabsorption. It is hoped to gain information on this and other points by following the changes in the yield and composition of the fluid at regular periods throughout the progress of pregnancy.

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CONTENTS.

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	PAGE
1. NEWTON, ROBERT. A comparative study of winter wheat varieties with especial reference to winter-killing	1
2. GOODEY, T. On the susceptibility of clover and some other legumes to stem-disease caused by the eelworm, <i>Tylenchus dipsaci</i> , syn. <i>devastatrix</i> , Kühn. (With Plate I)	20
3. SALAMAN, R. N. and LESLEY, J. W. Genetic studies in potatoes; sterility. (With Plate II)	31
4. CROWTHER, CHARLES and WOODMAN, HERBERT ERNEST. A study of nitrogen metabolism in the dairy cow	40
5. ARMSTRONG, S. F. The Mendelian inheritance of susceptibility and resistance to yellow rust (<i>Puccinia glumarum</i> , Erikss. et Henn.) in wheat	57
6. WOODMAN, HERBERT ERNEST and HAMMOND, JOHN. Note on the composition of a fluid obtained from the udders of virgin heifers	97

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THE CHEMICAL COMPOSITION OF ANIMAL BODIES

BY J. ALAN MURRAY, B.Sc.

University College, Reading.

(With 1 Text-figure.)

IN a previous communication (1) the author pointed out that the chemical composition of the bodies of farm animals is determined when the percentage of fat is known; for the composition of the non-fatty matter is practically the same in all, it is not affected by the "condition" (fatness) and it varies only to a slight extent with the age of the animals. The averages, in round numbers, deduced from Lawes and Gilbert's analyses (2) were as follows:

		Ash	Protein	Water
Young (growing) animals	...	4	20	76 $\frac{0}{0}$
Adults	6	22	72 $\frac{0}{0}$

These conclusions were necessarily more or less tentative in character because the data referred to only ten animals in all, viz., two pigs, three cattle and five sheep. More extensive data, recently to hand, afford a striking confirmation of the general thesis and enable us to determine the influence of age and the individual variation with greater precision.

Haecker (3) records separate analyses of the whole bodies of 49 individuals (cattle) ranging from about 100 lb. to 1500 lb. live weight and from 3.5 per cent. to 35 per cent. of body fat. Swanson (4) records separate analyses of the whole bodies of 36 individuals (pigs) ranging from about 20 lb. to 400 lb. live weight and from 5 per cent. to 60 per cent. of body fat.

In Haecker's experiments the animals were arranged in groups according to size with intervals of about 100 lb. average live weight between them. The mean values for each group are shown in Table I below.

The empty body weight is, of course, the live weight minus the refuse, i.e. the contents of stomach, intestines and urinary bladder. The fat-free empty weight is the weight of non-fatty matter in the actual body. It is found from the empty body weight by

$$m = M(100 - F)/100$$

or, directly from the live weight, by

$$m = M'(100 - F - R)/100,$$

Table I. *Composition of Whole Bodies (Cattle).*

No. in group	Live weight	Empty body weight	Fat-free empty weight	Fat in empty body	Composition of non-fatty matter			
					Ash	Protein	Water	P.A.
	Lb.	Lb.	Lb.	%	%	%	%	
5	107	90.0	86.4	4.00	4.44	20.72	74.84	4.69
4	207	163.2	153.5	6.01	4.71	20.36	74.93	4.33
4	301	246.1	218.5	11.19	4.83	21.13	74.03	4.37
5	416	339.5	303.8	10.55	4.86	21.59	73.55	4.15
5	504	418.1	360.7	13.73	4.89	22.19	72.91	4.55
3	614	493.7	424.5	13.97	5.33	22.58	72.08	4.46
4	708	587.8	490.2	16.57	5.36	22.31	72.33	4.15
3	815	692.1	563.8	18.52	5.20	23.08	71.72	4.43
3	905	774.5	587.6	24.08	5.18	23.27	71.25	4.24
4	1008	880.7	643.6	26.91	5.41	23.41	71.18	4.31
3	1108	976.3	663.8	32.03	5.62	24.13	70.25	4.29
3	1204	1077.0	728.8	32.32	5.46	23.66	70.88	4.33
4	1302	1150.5	776.6	32.50	5.60	23.40	71.00	4.18
1	1413	1237.0	834.1	32.58	5.21	23.95	70.84	4.60
1	1508	1352.9	843.9	37.59	5.14	25.19	69.66	4.90

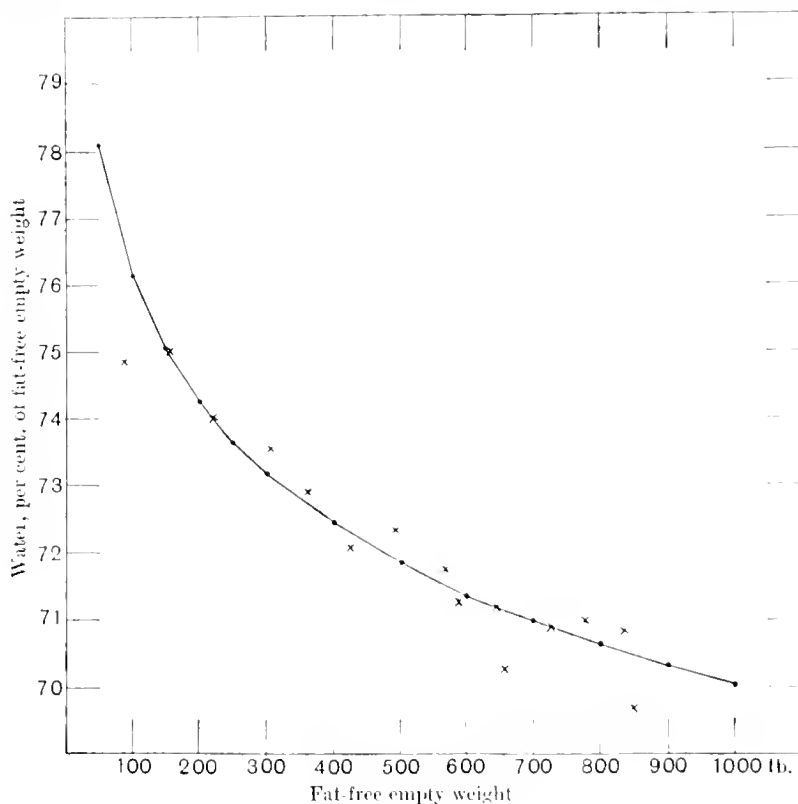


Fig. 1

where F and R are respectively the percentages of fat and refuse in the empty weight M and in the live weight M' respectively and m is the fat-free empty weight.

It will be seen, on reference to the table, that the percentage of water in the non-fatty matter diminishes progressively as age (weight) increases, and the percentages of ash and protein are correspondingly increased. There appears to be no consistent relationship between the ratio of protein to ash and the age of the animal; the variation observed must therefore be ascribed to individuality, i.e. to unknown causes. The mean value of the ratio P/A is $4.392 \pm 0.215^*$. In other words, protein forms from 79 per cent. to 84 per cent. of the dry substance of the non-fatty matter and ash the remaining 16 per cent. to 21 per cent. The mean percentages of these ingredients may therefore be found by the formulae

$$P = 0.815 (100 - W); A = 0.185 (100 - W)$$

where P , A and W are the percentages of protein, ash and water respectively in the non-fatty matter.

It only remains therefore to find an expression for determination of w . It is obvious that the relationship of water to weight is not one of simple proportion; but when the points are plotted out a definite order can be clearly discerned. The smooth curve in the diagram (Fig. 1) corresponds to the formula $w = 90m^{-.03624}$, where m is the fat-free body weight (lb.) and w is the percentage of water in the same. The points corresponding to the mean values of the observed data are indicated by \times , and it will be seen that the curve passes through or near to most of them. The mean deviation, $\frac{1}{N} \sum (d)$, (of all the individual cases) from the curve is 0.71.

In order to test these conclusions the composition of the three cattle analysed by Lawes and Gilbert was calculated from the live weight and percentage of fat and the results are given for comparison with the observed data in the table below.

The discrepancies between the observed and calculated results do not exceed the limits of individual variation in Haecker's data. In the case of the two oxen they are attributable mainly to deviation from the mean ratio of protein to ash. In the case of the calf they are attributable mainly to deviation from the curve of relationship of water to weight.

$$* \sqrt{\frac{1}{N} \sum (d^2)} = 0.319.$$

Table II. *Composition of Actual Bodies.*

	Fat calf		Half-fat ox		Fat ox	
	Calculated	Observed	Calculated	Observed	Calculated	Observed
Water	62.79	65.46	55.71	56.10	47.79	48.40
Protein	17.86	15.70	19.14	18.08	16.45	15.42
Ash	1.06	3.92	4.35	5.08	3.74	4.17
Fat	(15.29)	15.29	(20.80)	20.80	(32.02)	32.02
	100.00	100.07	100.00	100.06	100.00	100.01
Refuse		3.2		8.2		6.0
Fasted live weight (lb.)		259		1232		1419

The composition of the fattening increase (oxen) calculated from the data actually recorded by Lawes and Gilbert is as follows:

Fat 91.54%, Water 5.49%, Protein 0.6%, Ash 0.84%.

The percentages of water and protein in the increase might be regarded as an indication of growth, but as the animals were four years old it is improbable that any increase in size occurred, or that such increase, if it did occur, would be accompanied by a loss of 1.71 lb. of ash ingredients. Further, as the ratio of water to protein in the increase is 9/1 whereas in the whole body it is only 3/1 it is more reasonable to attribute the apparent increase in non-fatty constituents to difference in composition of the two animals. The individual variations revealed by Haecker's data are more than sufficient to justify this assumption. The inference therefore is that, in fully grown animals the fattening increase consists entirely of fat. This, of course, is acceptable on other grounds, but those who have maintained it have had to do so in the face of numerical data which could be quoted against it and of which, hitherto, no satisfactory explanation was forthcoming.

It is to be expected that in other animals the composition of the non-fatty matter will alter with age in much the same manner as in cattle, and the author anticipated no difficulty in tracing this relationship in pigs from Swanson's data. A preliminary survey, however, indicated that these results might be affected by the differences in feeding as well as by the age and individuality of the animals, and that the variations due to these combined causes would make it difficult clearly to distinguish the influence of any one. The best that could be done, it seemed, was to divide the data into three groups as shown in the table below.

It appears that the ratio of protein to ash in pigs is higher than in cattle, but the percentage of water in the non-fatty matter alters with age not only in the same manner but even in the same degree. The latter

inference, however, must be to a large extent discounted in view of the magnitude of the probable errors. It could not have been deduced from Swanson's data alone and until confirmed by further evidence it must be regarded as hypothetical.

Table III. *Influence of Age (Pigs).*

Fat-free empty weight	No. of animals	Composition of non-fatty matter					<i>P/A</i>
		Ash		Protein		Water	
Under 60 lb.	10	4.15	0.87	19.03	2.55	76.83 ± 3.12	4.60
60-100 "	10	3.91	0.36	19.89	1.35	76.02 ± 1.41	5.09
100-170 "	11	4.01	0.30	20.90	0.96	75.09 ± 1.04	5.21

The test of comparison with Lawes and Gilbert's data is not conclusive, but, so far as it goes, it indicates that the inference is perhaps more reliable than might be supposed. For this purpose the percentage of water in the non-fatty matter was calculated, as before, by the formula $w = 90m^{-.03624}$; but owing to the difference in the ratio of protein to ash different coefficients must be used for these ingredients, thus

$$P = 0.83(100 - W); A = 0.16(100 - W).$$

The results are as follows:

			Store pig		Fat pig	
			Calculated	Observed	Calculated	Observed
Water	58.28	58.15	42.71	43.01
Protein	14.28	14.45	11.13	11.35
Ash	2.86	2.82	2.22	4.72
Fat	(24.59)	24.59	(43.94)	43.94
Live weight (lb.)			—	94	—	185

In the following table the data are grouped according to the kind of food consumed by the animals.

Table IV. *Influence of Food (Pigs).*

Food	No. of animals	Empty weight	Fat-free empty weight	Fat in live weight	Composition of non-fatty matter			
					Ash	Protein	Water	<i>P/A</i>
		lb.	lb.	o o	o o	o o	o o	
Corn alone ...	5	80.27	47.45	36.69	3.56	17.93	78.51	6.03
Corn and ash ...	5	130.74	68.25	37.03	4.16	17.39	78.45	4.17
Corn and protein ...	10	217.94	109.31	48.01	3.54	21.34	75.12	6.07
Corn, ash and protein ...	5	226.74	118.57	45.55	4.41	21.17	74.42	4.82

In order to interpret these results correctly it is necessary to make allowance for the difference in size, i.e. to compare them with data

calculated for animals of the same size by means of the formula above, as follows:

	Corn alone		Corn and ash		Corn and protein		Corn, ash and protein	
	Cal'd	Obs'd	Cal'd	Obs'd	Cal'd	Obs'd	Cal'd	Obs'd
Water..	$\frac{a}{b}$ 78.25	$\frac{a}{b}$ 78.51	$\frac{a}{b}$ 77.21	$\frac{a}{b}$ 78.45	$\frac{a}{b}$ 75.91	$\frac{a}{b}$ 75.12	$\frac{a}{b}$ 75.68	$\frac{a}{b}$ 74.42
Protein	18.12	17.93	18.98	17.39	20.07	21.34	20.26	21.17
Ash	3.63	3.56	3.81	4.16	4.02	3.54	4.06	4.41

The correspondence between the observed and calculated data is, throughout, as close as could be expected in any circumstances. The inference therefore is that the differences which do occur should be attributed to individual variation and that the effect of the additions to the food was nil. It is to be observed, however (Table IV), that the addition of ash to the corn has reduced the ratio of protein to ash whereas the addition of protein has produced no appreciable alteration in that ratio. When ash and protein are added together the ratio is reduced only about half as much as by addition of ash alone. When the results are stated as percentages of the several ingredients these effects are obscured by the large amount of water present. Swanson remarks that the addition of protein and ash accelerates the growth of the animal but does not affect its composition.

The only data relating to sheep at present available are those of Lawes and Gilbert and, as these refer to only five individuals, they are inadequate for purposes of the present investigation. They may, however, be used to test the applicability of the formula deduced for cattle. Thus it is found that the ratio of protein to ash (4.3) is practically the same but the coefficient in the formula for water must be reduced, i.e. $w = 87m^{-.03624}$. Calculated on this basis the results are as follows:

Fat-free live weight (lb.)	59.63	70.82	73.84	74.20	123.80
Fat (per cent. of live weight)	28.59	23.59	18.70	35.60	45.80
Ratio of protein to ash	4.43	4.34	4.67	4.34	3.75
Water (per cent. of non-fatty matter)	...	observed	75.91	74.42	76.10	74.63	71.80
		calculated	75.46	74.56	74.46	74.42	73.07

AMOUNT OF BODY FAT.

In farm animals generally the ratio of fat to non-fatty matter varies within wide limits. It depends largely upon the quantity and quality of the food and, to that extent, it can be controlled. Elsewhere⁽¹⁾ the author has inferred that the maximum amount of body-fat is about

60 per cent. of the live weight. This estimate was based on the increase in basal katabolism, determined by Arncliffe⁽⁵⁾, in an ox. Pigs probably have a smaller capacity for food than ruminants of the same size; but, on the other hand, the food which is customary and suitable for them has a much higher productive index (ratio of dynamic to total energy). It was recognised that the result depends upon these factors and that any animal would require an indefinite time to attain the maximum degree of fatness of which it is capable under any given conditions. The fact that over 60 per cent. of body fat was recorded in two cases in Swanson's data indicates that the theoretical maximum is probably higher for pigs than for ruminants. At any rate it disposes of the objection that the estimated maximum (60 per cent.) is far beyond what had hitherto been recorded. The theoretical minimum of body fat is zero. The lowest recorded in the data under consideration is 5.27 per cent. in the pigs and 3.64 per cent. in the ruminants. There is no reason to believe that these records are the lowest attainable in living animals.

Attention may be called to the fact that the diminishing percentage of water in the non-fatty matter coincides with diminishing rate of growth and to the possible connection between these phenomena. The biological significance of the constants in the formula for water may also prove a matter of interest to physiologists.

SUMMARY.

Animal bodies are composed of fat and non-fatty matter. The relative proportions of these two ingredients vary within wide limits but can be controlled by food. The non-fatty matter consists of water, protein and ash. The percentage of water varies with the age of the animal in a definite manner. This has been determined with tolerable certainty for cattle. The available evidence indicates that the same formula is applicable to pigs and, with slight modification, also to sheep, but these inferences require confirmation. The ratio of protein to ash is the same in sheep as in cattle but in pigs it is higher. In any case it does not alter with the age of the animal but it may be influenced to a certain extent by the food. Individual variation is wider in pigs than in ruminants. The average composition of the whole body at any stage can be calculated when the live weight and percentage of fat in it are known.

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THE EFFECT ON THE PERCENTAGE COMPOSITION OF THE MILK OF (a) VARIATIONS IN THE DAILY VOLUME AND (b) VARIATIONS IN THE NATURE OF THE DIET.

BY WILLIAM TAYLOR, M.D., D.P.H. (*Carnegie Research Scholar*),
AND ALFRED D. HUSBAND, A.I.C.

(*From the Rowett Research Institute, Aberdeen.*)

(With 4 Text-figures.)

THE study of lactation has almost always been undertaken from one of two very diverse points of view, the physiological and the commercial. The chief aim of the physiologist has been to determine, either the origin of the various constituents of the milk and the method of their elaboration, or the extent to which internal secretions affect the flow of milk by their initiation, inhibition or stimulation of milk secretion. From the commercial or agricultural point of view, on the other hand, the chief interest has centred round the problem of the production of butter fat, and a very large number of experiments have been conducted to determine by what method of feeding a cow could be caused to give the maximum yield of milk with the highest percentage of fat.

Much of this work is of great value, but in many cases the fat is the only constituent of the milk which has been determined separately, the other constituents having been estimated together as milk solids other than fat.

With regard to the diets, unless the calorific consumption is stated, a simple addition of extra protein or fat to a ration does not allow of a judgment being formed as to whether the alteration in the daily volume and percentage composition of the milk is due to protein or fat *per se*, or simply to an increase in the calorific value of the ration. This consideration would appear to have been lost sight of in some cases.

The most complete investigation of the influence on the percentage composition of the milk of feeding with an excess of one of the energy-yielding constituents of the food is that of Voit⁽¹⁾, who worked with a bitch. He found that, while an excess of one constituent in the food

tended to give a slight increase in the percentage of that constituent in the milk, the deviation from the normal was comparatively slight.

The work done since Voit's time has produced rather contradictory results. For example, Ingle(2) found that a protein rich diet increased both the yield of milk and the percentage of fat, while Crowther(3) found that a food rich in protein gave a decrease in the yield but an increase in the fat content. Again, Jordan and Jenter(4) show that the amount of fat in the food is without influence on the percentage of fat in cows' milk, while Morgan, Berger and Fingerling(5) found that a fat poor diet produced in goats a milk with a low percentage of fat.

There is difficulty in correlating contradictory results such as these, and the difficulty is increased where there is uncertainty as to the calorific value of the food digested and absorbed, and, further, when no account has been taken of the probable influence on the percentage composition of a change in the daily volume of milk secreted. In the voluminous literature on milk there is very little reference to the influence of volume on composition—except in the case of the fat—and it is possible that this may explain some of the contradictory results obtained by different workers on the question of the influence of different diets.

PRESENT INVESTIGATION.

The present investigation was undertaken to determine:

A. To what extent variations in the daily volume (or rate of secretion) of the milk were accompanied by variations in its percentage composition.

B. To what extent the percentage composition of the milk could be influenced by diet.

The experimental animal was the goat, and throughout the investigation the milking was done at 9 a.m. and 5 p.m. The total volume of the 24 hours' secretion of milk was measured, mixed and analysed daily, the percentages of total protein, casein, albumin plus globulin, non-protein nitrogen, fat, lactose and ash being determined.

METHODS OF ANALYSIS.

Total Protein. The total nitrogen was estimated by the Kjeldahl method, the protein factor 6.38 being used for the determination of total protein.

Casein. The casein was precipitated with acetic acid and filtered off, after which the nitrogen was determined by the Kjeldahl method, the same protein factor, 6.38, being made use of.

Albumin and Globulin. These were precipitated by tannic acid from the filtrate obtained in the estimation of casein, after which they were estimated together as described above for casein.

Non-Protein Nitrogen. This was estimated by the difference between the percentage of total protein and the sum of the percentages of casein, albumin and globulin.

Fat. By the Soxhlet method.

Lactose. Fehling's method and Benedict's method were both made use of; but in the individual experiments one method was adhered to throughout.

Ash. By the ordinary method of ignition.

EXPERIMENTAL.

A. THE EFFECT OF VARIATIONS IN VOLUME ON THE PERCENTAGE COMPOSITION OF THE MILK, WITH AN ANIMAL ON A DIET OF CONSTANT COMPOSITION.

Experiment I. (Analytical Data, Table I.)

In this experiment a series of milk analyses was carried out from day to day in the case of a goat fed on a diet of hay and a mixture of meals made from locust beans, earth nuts and oats, this mixture being sweetened with sugar. The animal was allowed to eat to its appetite, but the nature of the diet was constant throughout. Owing to the confinement, the monotonous diet and the advanced stage of lactation, the volume of the milk fell steadily throughout the experiment with, as a rule, but slight variations from day to day.

Table I.

	Volume	Protein	Casein	Albumin + globulin	Non- protein nitrogen	Fat	Lactose	Ash
	c.c.	%	%	%	%	%	%	%
1	310	4.50	3.07	1.20	0.23	4.54	4.26	0.98
2	290	4.73	3.38	1.14	0.21	4.85	4.28	0.97
3	320	4.50	3.12	1.15	0.23	5.24	4.24	0.98
4	310	4.39	3.01	1.15	0.24	5.80	4.21	0.94
5	255	4.59	3.19	1.16	0.23	5.55	4.19	0.94
6	250	4.72	3.34	1.20	0.18	5.12	4.24	0.96
7	245	4.82	3.37	1.24	0.21	5.56	4.24	0.98
8	240	4.86	3.41	1.24	0.21	5.62	4.35	0.97
9	225	5.00	3.54	1.28	0.19	5.38	4.30	1.00
10	235	4.89	3.39	1.28	0.21	5.76	4.26	0.98
11	185	5.25	3.70	1.35	0.20	5.56	4.21	1.00
12	190	5.17	3.63	1.36	0.19	6.01	4.30	1.02
13	220	4.82	3.39	1.25	0.18	6.86	4.26	0.97
14	240	4.54	3.14	1.24	0.16	6.37	4.26	1.08
15	170	5.01	3.52	1.25	0.25	6.30	4.26	0.98
16	145	5.40	3.79	1.36	0.24	5.94	4.30	1.04

Hammond and Hawk (6), and others, have shown that an inverse relationship exists between the percentage of fat and the daily volume. This was well brought out, but it may be seen from Fig. 1 that the percentage of protein also varied inversely as the volume with a regularity at least equal to that of the fat, though the extent of the variation was less marked. The percentage of lactose maintained a very constant level, varying between 4.19 per cent. and 4.30 per cent. The percentage of ash, which varied from 0.91 per cent. to 1.08 per cent., may be seen from the analytical data to have shown a tendency to rise with the fall in volume towards the end of the experiment.

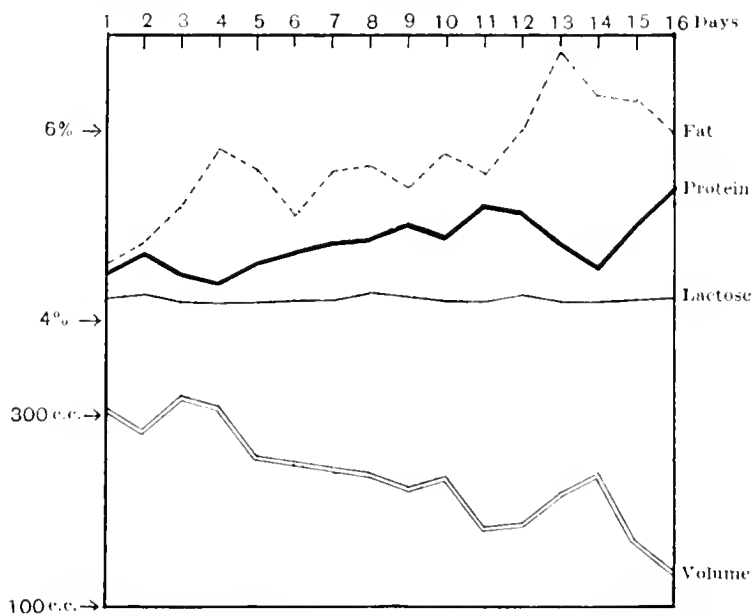


Fig. 1. Showing the effect of variations in volume on the percentage composition of the milk.

Experiment II. (Analytical Data, Table II.)

The fluctuations in volume from day to day having been comparatively slight in the preceding experiment, advantage was taken in this case of the decrease in yield brought about by an absence of food, and the increase which follows the resumption of feeding.

A goat was fed for eight days on an unrestricted diet of hay and oatmeal, and on the 9th and 10th days no food was taken. From the 11th day onward the feeding was resumed.

Table II.

	Volume c.c.	Protein %	Fat %	Lactose %	Ash %
1	500	2.97	3.95	4.33	0.82
2	480	3.09	4.32	4.07	0.84
3	460	3.07	4.08	4.12	0.88
4	470	3.00	4.09	4.00	0.87
5	470	2.91	4.45	4.00	0.89
6	460	2.95	4.47	4.07	0.79
7	460	2.98	4.53	4.07	0.83
8	460	2.93	3.95	4.07	0.79
9	350	3.02	5.17	3.80	0.80
10	50	9.24	10.16	2.31	1.37
11	340	2.88	5.40	4.00	0.98
12	350	2.52	4.81	4.23	0.91
13	320	2.93	5.53	4.20	0.94
14	230	3.23	5.98	4.21	0.96
15	275	2.93	5.71	4.05	0.89
16	300	2.77	5.16	3.82	0.90
17	275	2.97	4.97	4.12	0.92

The following extract from the analytical data brings out very clearly the inter-relationship of volume and composition.

	Volume c.c.	Protein %	Fat %	Lactose %	Ash %
Last day before starvation ...	460	2.93	3.95	4.07	0.79
Second day of	50	9.24	10.16	2.31	1.37
„ after	350	2.52	4.81	4.23	0.91

It will be seen that with the great fall in volume on the second day of starvation there was a rise in the percentages of all the constituents of the milk, with the exception of the lactose, the percentage of which came down with the volume.

The percentage of fat, as was expected, showed a marked rise, but the percentage of protein was found to rise markedly also; and the percentage of ash, although affected to a less extent, distinctly increased with the great and sudden fall in the volume of milk.

The lactose, a constituent of the milk the percentage of which is normally very constant, showed, on the other hand, a percentage decrease as definite as the percentage increase in the protein, fat and ash.

With the renewed consumption of food the daily volume of milk increased, with, at the same time, a close approximation to its previous percentage composition.

Experiments III and IV. (Analytical Data, Tables III and IV.)

These experiments were conducted to determine whether the inter-relationship of volume and composition, maintained during Exps. I and II, would still hold good during the physiological increase and decrease

in volume which takes place at the commencement and cessation of lactation respectively.

Table III.

	Volume	Protein	Casein	Albumin + globulin	Non- protein nitrogen	Fat	Lactose	Ash
	c.c.	$\frac{o}{o}$	$\frac{o}{o}$	$\frac{o}{o}$	$\frac{o}{o}$	$\frac{o}{o}$	$\frac{o}{o}$	$\frac{o}{o}$
1	130	5.98	3.02	2.66	0.30	8.07	3.65	0.81
3	250	4.11	2.55	1.37	0.19	5.56	4.39	0.79
5	330	1.07	2.59	1.31	0.17	5.91	4.70	0.81
7	365	3.88	2.45	1.25	0.18	5.70	4.72	0.78
9	400	3.65	2.44	1.05	0.16	6.08	5.02	0.68
11	500	3.81	2.49	1.08	0.18	5.18	5.10	0.76
13	430	3.62	2.29	1.03	0.30	5.22	5.06	0.75

Table IV.

	Volume	Protein	Casein	Albumin + globulin	Non- protein nitrogen	Fat	Lactose	Ash
	c.c.	$\frac{o}{o}$	$\frac{o}{o}$	$\frac{o}{o}$	$\frac{o}{o}$	$\frac{o}{o}$	$\frac{o}{o}$	$\frac{o}{o}$
1	100	5.36	4.26	0.93	0.17	5.53	4.06	0.92
2	340	5.65	4.55	0.98	0.12	6.02	4.06	0.96
3	275	6.17	4.91	1.05	0.21	6.53	4.24	0.96
4	250	6.52	5.25	1.11	0.13	7.83	4.16	0.99
5	210	6.66	5.30	1.20	0.16	7.20	4.05	1.01
6	185	7.14	5.59	1.39	0.16	7.96	4.05	1.02
7	110	8.08	6.38	1.55	0.15	8.86	4.16	1.12
8	80	8.61	6.84	1.66	0.11	8.63	3.71	1.13
9	55	8.47	6.61	1.73	0.13	9.70	3.02	1.08
10	35	9.96	7.56	2.28	0.12	11.36	3.07	1.30
11	45	10.46	7.64	2.61	0.21	10.94	2.27	1.33
12	35	8.96	6.50	2.45	0.01	9.31	2.62	1.19
13	30	9.22	6.68	2.57	?	8.40	2.89	1.20
14	20	9.20	4.90	4.20	0.10	9.44	2.35	1.36
15	25	9.06	5.88	3.06	0.12	9.06	2.27	1.18
16	15	9.88	6.64	3.21	0.03	9.08	2.20	1.36
17	15	10.88	7.28	3.56	0.04	9.49	2.20	1.42
18	5	11.20	7.64	3.56	?	7.65	—	1.41

It may be seen from Figs. 2 and 3 that the changes in the percentage composition of the milk which accompanied alterations in volume, due to these causes, were the same in nature as the changes which accompanied the fluctuations in volume brought about in the preceding experiment by the two days' starvation.

(During Exp. III, commencement of lactation, a milk analysis was carried out only on alternate days.)

As a result of these and other similar experiments, not described here for reasons of economy of space, it seemed possible to formulate the following general principle: That, on a diet of constant composition, the percentages of all the constituents of the milk, with the exception of the lactose, tend to vary inversely as the daily volume of milk secreted:

and that the percentage of lactose, while normally very constant, tends to vary directly as the volume, this tendency being particularly apparent at the beginning and end of lactation.

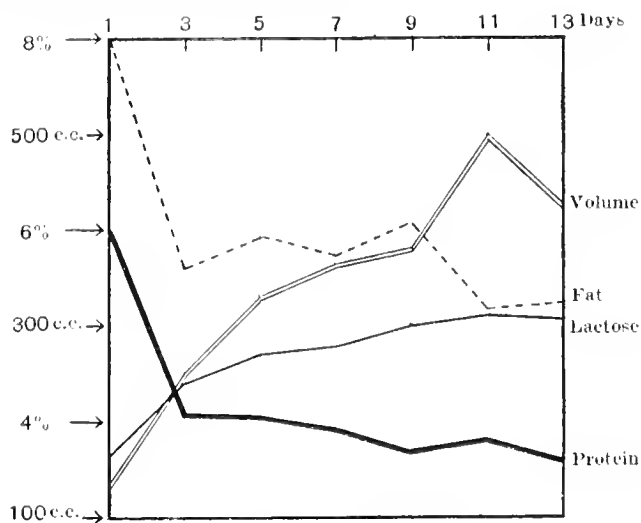


Fig. 2. Beginning of Lactation.

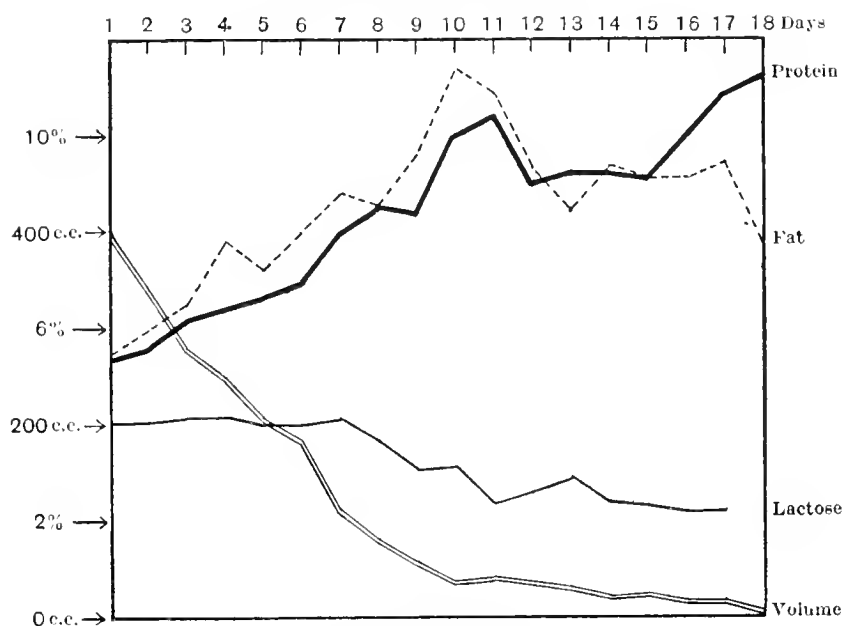


Fig. 3. End of Lactation.

B. THE INFLUENCE OF DIET ON THE VOLUME AND PERCENTAGE COMPOSITION OF THE MILK.

Experiment V. (Analytical Data, Table V.)

The above principle having been established on a diet of constant composition, it was desired to see to what extent it might be departed from on diets which were abnormally high in some one of the three energy-yielding constituents of the food, viz., protein, fat and carbohydrate.

Table V.

(Average daily volume and average percentage composition of the milk for each dietary period.)

Nature of Diet	Volume c.c.	Protein %	Casein %	Albumin Non protein		Fat %	Lactose %	Ash %
				globulin %	nitrogen %			
High fat (14 days)	395	4.14	3.20	0.94	0.20	4.40	4.23	0.96
Normal (9 days)	319	4.66	3.39	1.27	0.23	5.33	3.90	1.10
High protein (16 days)	533	4.34	3.23	1.11	0.39	3.23	1.05	0.93
Normal (5 days)	380	4.42	3.43	0.99	0.26	4.40	3.91	1.08
High carbohydrate (13 days)	439	4.12	3.34	1.08	0.16	3.52	4.17	0.92

(In calculating the average percentage of protein the non-protein nitrogen was not included.)

To do this the normal calorific intake of the goat was first estimated by feeding her for 20 days on an unlimited and congenial diet of known composition, weighing the amount of food left over at the end of each day, and finally calculating the average daily calorific consumption. On this basis each of the experimental diets was made up.

In this experiment, a high fat, a high protein and a high carbohydrate diet were given, a period of normal diet intervening between each period of special diet. The average daily volume of the milk has been worked out for each dietary period, and against this has been set out the average percentage composition of the milk for the corresponding period. The first two days after each change of diet have, however, been omitted in the calculation of these averages. For reasons of space the results of the individual milk analyses from day to day have not been given in the tables, but only the average daily volume and average percentage composition for each dietary period. (For details of diets, see Table VI.)

Table VI.

High Fat Diet.

Hay	525 gms.)	} = {	Protein	121.7 gms.)	} = 4225 calories.
Earth nuts	243 ..		Fat	136.2 ..	
Oatmeal	102 ..		Carbohydrate	599.9 ..	
Locust bean meal	266 ..				
Sugar	71 ..				

High Protein Diet.

Hay	560 gms.)	} = {	Protein	305.1 gms.)	} = 3702 calories.
Plasmon	300 ..		Fat	41.2 ..	
Oatmeal	335 ..		Carbohydrate	504.5 ..	

High Carbohydrate Diet.

Hay	586 gms.)	} = {	Protein	105.3 gms.)	} = 4633 calories.
Locust bean meal	374 ..		Fat	40.3 ..	
Maize meal	531 ..		Carbohydrate	933.2 ..	
Oatmeal	98 ..				

The result of this experiment can most clearly be seen by consulting Fig. 4. In this graph the horizontal lines represent average volumes and average percentages, the sloping lines connecting the various horizontal levels representing the two days at the beginning of each dietary period which have been left out in the calculation of the averages.

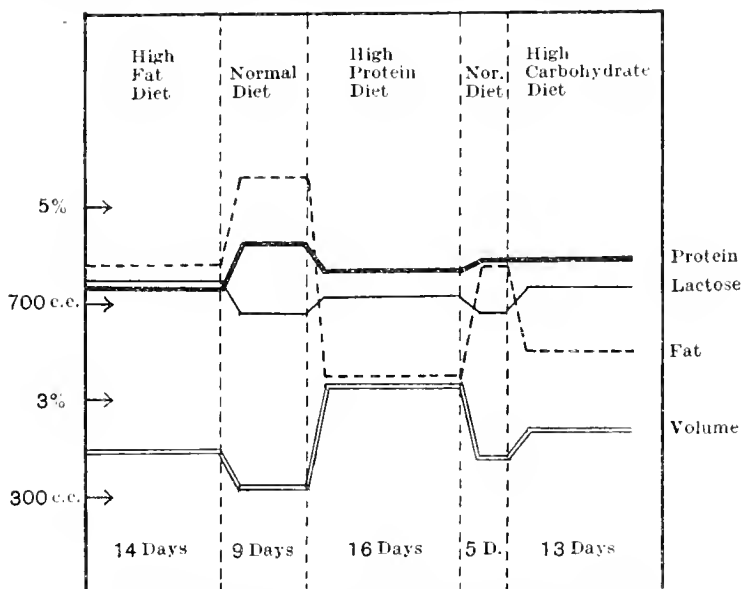


Fig. 4. Showing the effect of diets of different composition on the volume and percentage composition of the milk.

It will be seen that in no case was there a direct increase in the percentage of that constituent of the milk corresponding to the constituent of the diet which was present in excess. The protein, fat and ash all tended to vary, albeit to different degrees, inversely, and the lactose directly, as the daily volume of milk secreted. It is true that with the different diets variations in the percentage composition of the milk were produced, but these variations were of such a nature, and took place to such a degree, as could be accounted for by the variations in the rate of secretion, and, therefore, in the daily volume of the milk.

Fig. 4 brings out very clearly the direct relationship existing between the percentage of lactose and the daily volume of milk secreted.

Several different feeding experiments were carried out with, in every case, results similar to those stated above. In one of these it was considered that a high protein diet had markedly stimulated the rate of milk secretion. In this experiment, therefore, it is worthy of note, the high protein diet was made up in such a way that it was of lower calorific value than either the high fat diet which preceded it, or the high carbohydrate diet which followed it. The greatest secretion of milk, however, was got on the diet of lowest calorific value, viz., the high protein diet. The following are the main features of the diets set out against the average daily volumes of milk obtained.

		Calorific value of diet	Volume of milk
High fat diet	...	4230 calories (containing 136 gms. of fat)	400 c.c.
High protein diet	...	3700 calories (containing 305 gms. of protein)	540 c.c.
High carbohydrate diet		4630 calories (containing 933 gms. of carbohydrate)	440 c.c.

There is one constituent, however, not *of* the milk but *in* the milk, the percentage of which was found to have a direct relationship to diet, viz., the non-protein nitrogen. This was estimated by the difference in the figures for the percentage of total protein and the sum of the percentages of casein, albumin and globulin. While not protein, therefore, it is expressed separately in terms of protein, in view of the fact that it was included in the estimation of nitrogen from which the percentage of total protein was calculated. It was found to vary from about 0.1 per cent. to 0.4 per cent. with the amount of protein taken in the food. (This is being dealt with in another publication.)

DISCUSSION OF RESULTS.

It seems justifiable to conclude from the results of these experiments, that with the exception of the non-protein nitrogen, which is not a product of the mammary gland, diet has no direct influence on the percentage composition of the milk. It has, however, an indirect influence by reason of its effect on the daily volume, but the percentages in which the various constituents of the milk will be present in any particular daily volume, obtained by some special method of feeding, will be the same as those in which they would be present in the same daily volume were it obtained in the ordinary course of lactation.

Indeed, the inverse relationship of percentage of fat and daily volume is so unvarying *on the average*, as Tocher(7) points out in the case of the cow—in his statistical analysis of milk records, where he deals with the milk of thousands of cows—that it may be stated, and illustrated by a graph, with the mathematical precision of a proposition in Euclid. He says: "It will be seen that there is a direct proportional relationship between quantity and average quality.

"Given two known values of average quality for any two types of quantity, a straight line joining the two values of average quality and extended on either side will give the average qualities for all other types of quantity, average quality being represented in value by a straight line proportional to its value standing at right angles to the base line of quantity at a point corresponding to its appropriate type of quantity."

By the term "quality" in the above statement is understood, of course, the percentage of fat.

While this is true of the fat it is probably no less true of the protein. Throughout the present investigation the percentage of protein in the milk and the daily volume varied inversely with a regularity at least equal to that of the fat. It is true that these percentage variations were not so marked as in the case of the fat, the inverse rise and fall with each variation in volume having been less, as a rule, but they took place from day to day with an equal consistency if to a less degree, even during periods of special dieting.

The probability is, therefore, that it will be found from a statistical analysis of milk records—when the requisite data become available—that the relationship of average percentage of protein to each particular daily volume can be formulated as clearly and precisely in the case of this constituent of the milk as has been done by Tocher in the case of the fat. It is true that in a graph the line representing the fat and that

representing the protein would be at different angles, as the protein neither rises nor falls with variations in volume to the same extent as the fat, but the likelihood is that where Tocher uses the word "quality" the words "percentage of protein" might be substituted, without impairing the accuracy and applicability of the statement.

During the present investigation the individual protein constituents were estimated as casein on the one hand, and albumin plus globulin on the other, and it was found that, from day to day, the casein and albumin plus globulin were present in varying proportions. Sometimes, when there was a rise in the percentage of protein, it was found that this was due chiefly to the casein; and again, on some other occasion, the albumin and globulin would be found to have contributed largely to the rise. Despite these irregularities, however, it was found that there was a tendency for the casein on the one hand, and the albumin plus globulin on the other, to maintain individually, as well as in combination, an inverse percentage relationship to volume; and, consequently, these protein constituents of the milk were found present in larger percentages at the beginning and end of lactation than during the period when it was at its height.

With regard to the ash, this was found to be the least variable of the constituents of the milk, but the analytical data for Exps. II and V show that where there was a marked fluctuation in volume the percentage of ash showed an inverse relationship to it.

The relationship of lactose to volume is not so apparent as that of the protein or fat. Crowther and Ruston(s) say: "It will be seen that the percentage of sugar, after rising a little with the early stages of lactation, fell steadily throughout the rest of the period." While due credit must be given for this observation it may be pointed out that the percentage of lactose is only indirectly connected with the period of lactation. Its real relationship is to volume, and the accuracy of the words "steadily throughout" would seem to depend on the evenness with which the volume rises at the commencement of lactation, the evenness with which it is maintained, and the evenness with which it falls at the end of lactation. During the present investigation, where there were wide variations in volume at the height of lactation, due either to dieting or starvation, the percentage of lactose was found to vary also, and showed a direct relationship to it—as may be seen in Fig. 1.

The inter-relationship of volume and composition suggests a theory of milk secretion which may be put forward here, and on which further work is being carried out.

Van der Laan(9) shows that a state of osmotic equilibrium exists between the blood and the milk, and that this equality of osmotic pressure persists even in diseases of the udder. As lactose is the substance which is likely to play the most considerable part in determining the osmotic pressure in the milk, the osmotic pressure in the blood is likely to be expressed to a very great degree by the percentage in which this constituent is present in the milk; and as, normally, the osmotic pressure in the blood must be considered to be a constant, with but slight variations from day to day, it is to be expected that an equal constancy will prevail in the percentage in which the lactose is present in the milk. It has been found that, while the percentage of lactose tends to vary directly as the daily volume, at the height of lactation these variations are normally so slight that an approximately constant percentage is maintained. The conclusion to be drawn is not that the lactose is elaborated till it reaches this practically constant percentage, but that it is kept down to this constant level by the rate of secretion of the milk. The suggested theory of secretion is, therefore, that the elaboration of lactose produces a flow of milk by a process of osmosis. This would explain why, at the height of lactation, the percentage of lactose varies but slightly and tends to do so directly as the daily volume, at the same time reducing the percentages of all the other constituents of the milk by a process of dilution—hence their inverse percentage relationship to volume.

CONCLUSIONS.

1. The percentage composition of the milk seems to be determined by its rate of secretion.
2. The percentages of protein, fat and ash vary inversely, and the percentage of lactose varies directly, as the daily volume, the greatest variation being shown by the fat and the least by the inorganic elements.
3. There is an inverse relationship between the percentage of lactose and the percentages of all the other constituents of the milk, this being particularly apparent in the case of the fat.
4. Diet has no direct influence on the percentage composition of the milk, except in the case of the non-protein nitrogen which is not a product of the mammary gland. Diet has, however, an indirect influence by reason of its effect on the daily volume.
5. A high protein diet would appear to stimulate the rate of secretion of the milk.

6. It is suggested that the quantity of lactose elaborated by the mammary gland controls the daily volume of the milk, and that, therefore, the rate of its elaboration controls the rate of milk secretion.

We wish to express our indebtedness to Dr J. B. Orr, who suggested this research, for constant encouragement and advice during the progress of the work.

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THE CITRIC SOLUBILITY OF MINERAL PHOSPHATES

BY J. F. TOCHER, D.Sc., F.I.C.

*Consulting Chemist to the Highland and Agricultural Society of Scotland;
Lecturer on Statistics, University of Aberdeen.*

(With 8 Diagrams.)

I. INTRODUCTORY.

THE Fertilisers and Feeding Stuffs Act 1906, Section 10, defines the expressions "soluble" and "insoluble" to mean that the fertilising constituent is soluble or insoluble in water, or, if specified in the invoice, to mean that the fertilising constituent is soluble to the extent guaranteed in a solution of citric acid, or other solvent, of the prescribed strength. In particular the section defines the percentage of soluble phosphate and the percentage of insoluble phosphate to mean respectively the percentage of tribasic phosphate of lime equivalent which has been, or that which has not been, rendered soluble. "Citric solubility" under the Act is further and more definitely defined in the Fertilisers and Feeding Stuffs General Regulations 1906 as follows:

"When, in an invoice relating to *basic slag* or *basic superphosphate*, it is specified that a certain percentage of the phosphate contained in the basic slag or superphosphate is soluble in citric acid, this shall be taken to mean that it is capable of being dissolved to the extent of such percentage when 5 grams of the fertiliser and 500 cubic centimetres of water, containing 10 grams of citric acid, are continuously agitated in a flask or bottle of about 1 litre capacity for the period of half an hour at the ordinary temperature."

It is clear from these regulations that "citric solubility" refers to basic slags and "basic superphosphates"¹ and that the citric solubilities of basic slags and of basic superphosphates have to be determined at "room" temperature by means of a 2 per cent. solution of citric acid, the duration of contact being limited to half an hour in a quantity of

¹ The seller need not, unless he chooses to do so, give any guarantee of citric solubility.

the fertiliser exactly one half in amount of citric acid present. The basis of this test is evidently the work of Wagner and appears to be of a wholly empirical character. Since Wagner's method has been adopted as an official test, citric solubility has been studied by a number of different workers. Stead¹ found that for normal basic slags the solubility increased with the amount of silica present. He also showed that citric solubility was associated with the degree of fineness of the powder. Robertson² studied the degree of solubility of mineral phosphates in citric acid using the official test. He shows that mineral phosphates are completely soluble in 2 per cent. citric acid solution if a sufficient number of extracts are made by successive half hour contacts. Robertson in his conclusions states that "Even a small amount of free lime or calcium carbonate decreases substantially the solubility of mineral phosphates as judged by the citric acid test. When a large amount of calcium carbonate or free lime is present, the citric acid test as commonly practised, is a test for lime and not for phosphates. It is important in this respect to distinguish between free lime and calcium carbonate, and lime actually entering into the composition of the phosphate. The higher the percentage of lime actually entering into the phosphate compound, the higher the citric solubility of the phosphate." He has also shown that fluor-spar greatly decreases citric solubility in slag and concludes that the official test gives no true idea of the solubility of the phosphate in slag. He states that "one of the effects of fluor-spar is to cause the formation of a phosphate which does not contain silica in combination as is the case with high citric soluble slags." The effect of fusing with fluorides apparently is that a compound of silicon and fluorine is formed leaving lime and phosphorus in combination. Dixon³ made a study of the citric solubility of various bone phosphates. In his case he varied the citric acid concentrations and with a constant weight of fertiliser he found that in every case the stronger the citric acid solution the greater was the amount of phosphate dissolved. Ramsay⁴ prepared pure tricalcium phosphate by mixing three equivalents of CaO with one equivalent of P₂O₅ and showed that 91 per cent. of the total phosphoric acid content of the pure tricalcium phosphate was soluble in the prescribed 2 per cent. citric acid solution in 30 minutes. He also showed that the simple addition of calcium carbonate reduced citric solubility. Russell and Prescott⁵ studied the citric solubility of the phosphates of the soil. They

¹ *Trans. Faraday Soc.* **16**, Part 2.

² *Soc. Chem. Ind.* No. **4**, **35**.

³ *Journ. of Agric. Sci.* 1906, Part 4.

⁴ *Ibid.* June, 1917.

⁵ *Ibid.* Sept. 1916.

found a greater solubility for short periods of contact when compared with long periods and established the fact that adsorption of phosphate took place during long periods of contact.

II. CITRIC SOLUBILITY—OFFICIAL AND OTHERWISE.

The writer was attracted to this subject by the fact that the citric solubility of certain commercial phosphates was determined in a dilute citric acid solution (0·2 per cent.) and not by means of the 2 per cent. solution prescribed by the official test for slags.

Samples of ground mineral phosphate are occasionally guaranteed to contain as much as 50 per cent. "citric soluble" phosphate. To the unwary this might be taken to mean that the sample contained 50 per cent. "citric soluble" phosphate as determined by the official test for slags and "basic superphosphates" prescribed in the Regulations. As a matter of fact, however, the citric solubility in this case was determined for the sellers by agricultural analysts by agitating for half an hour 5000 parts of a solution of citric acid (0·2 per cent. strength) with 1 part of the sample. That is to say the amount of the citric acid employed was one-tenth of the amount officially prescribed while the proportion of fertiliser was 50 times less than the proportion prescribed as may be seen from the following table (Table I).

Table I.

Test	Citric acid	Fertiliser	Total volume
Official quantities	10 grams	5·0 grams	500 c.c.
Quantities for private test ...	1 gram	0·1 gram	500 ..
i.e.	10 grams	1·0 ..	5000 ..

The following results (Table II) were obtained on using the official citric solubility test on samples of ground mineral phosphate and basic slag:

Table II.

Basic slags				Mineral phosphate			
Citric sol. phos.	Total phos.	Fineness	Percent. of total phos. dissolved	Citric sol. phos.	Total phos.	Fineness	Percent. of total phos. dissolved
27·94	30·59	84·8	91·3	21·92	64·86	64·7	33·8
28·21	31·00	79·0	91·0	18·26	55·86	85·1	32·7
4·82	19·35	76·0	24·9	20·39	57·84	84·5	35·3
6·36	25·55	74·1	24·5	19·72	57·98	82·9	34·0
22·68	23·60	93·6	96·1	21·53	58·51	98·0	36·8
28·54	39·10	85·4	73·0	19·36	61·92	88·0	31·3

The results of this table show that when mineral phosphate is as finely ground as basic slag a fair amount of phosphate is rendered "citric soluble" by the official method but the "citric solubility" of mineral phosphates appears generally to be much less than the citric solubility of slags, when this test is applied.

III. THE SCHEME OF EXPERIMENTAL WORK.

The citric solubility of mineral phosphates was accordingly studied at "room" temperature over a period of 30 minutes agitation:

(1) In varying dilution, the quantities of acid and mineral phosphate being constant.

(2) In varying concentrations of acid, the volume of fluid and the weight of mineral phosphate being constant.

(3) With varying amounts of the phosphatic fertiliser, the volume of the fluid and the concentration of acid being both constant.

In order to compare the citric solubility of mineral phosphates under the above conditions, with the citric solubility of a pure phosphatic compound, a fourth series of experiments was conducted, namely,

(4) the solubility of dicalcium phosphate in dilute hydrochloric acid.

It is evident that whatever be the value of determining the proportion of citric soluble phosphate in a phosphatic fertiliser, it is necessary, for comparative purposes, if other conditions are similar, that the test should be applied in the same way and with the same proportions of citric acid or of other acid and of fertiliser as prescribed officially, for basic slags and basic superphosphates.

Other conditions are, of course, open to study. For example we could have two of the above factors varying differently with a series of constant values for the third factor. The citric solubility of tricalcium phosphate and other pure phosphatic compounds could be studied and the results compared with the results from the above four series. The writer has not been able to carry out these latter experiments. He therefore submits the results of experimental work under the above four heads¹.

¹ The writer has to acknowledge his indebtedness to Mr John E. Ritchie, M.A., B.Sc., A.I.C., who has performed the necessary analytical determinations in the experiments on mineral phosphates, and has also given valuable assistance in preparing the memoir. He has also to thank Mr W. T. H. Williamson, B.Sc., A.I.C., for the determinations in the case of dicalcium phosphate.

IV. EXPERIMENTS WHERE THE QUANTITIES OF MINERAL PHOSPHATE AND CITRIC ACID USED WERE CONSTANT AND THE DILUTION WAS VARIED.

In order to determine the variability in citric solubility of mineral phosphate with varying dilutions of citric acid, a series of experiments was conducted with different dilutions shaking for half an hour, the time prescribed in the official test. The quantities of citric acid and of mineral phosphate used are indicated in the following table (Table III). Let m_1 = amount of citric acid used, m_2 = amount of mineral phosphate used, and m_3 = volume of fluid used. In this series the ratio m_1/m_2 was made constant (and equal to 2) in order to secure that the effect of the presence of $\text{Ca}(\text{OH})_2$ and of CaCO_3 and other hydrates and carbonates, on the citric acid concentration was of a constant character. Throughout this series of experiments 10 grams citric acid and 5 grams mineral phosphate were used, the volume being varied as shown in Table III.

Table III. *Quantities of mineral phosphate and citric acid used are constant—dilution, i.e. degree of concentration, varies.*

{ 5 grams mineral phosphate
10 grams citric acid

1 Exp.	2 Vol. of sol.	3 4 Wt. of Phosphate dissolved. Grams per volume stated		5 Phosphate dissolved per cent. of sample as $\text{Ca}_3(\text{PO}_4)_2$	6 7 Percentage dissolved of total phosphate content		8 9 Molecular concentration of phosphate at end of 30 minutes. Gram-mols. per litre	
		Observed as $\text{Ca}_3(\text{PO}_4)_2$	Theory as $\text{Ca}_3(\text{PO}_4)_2$		Observed	Theory	Observed	Theory
1	500	0.9620	0.9697	19.24	29.8	30.1	0.006206	0.006256
2	625	1.0210	1.0215	20.42	31.7	31.7	0.005270	0.005272
3	833	1.0875	1.0907	21.75	33.7	33.8	0.004210	0.004222
4	1250	1.2080	1.1990	24.16	37.5	37.2	0.003117	0.003094
5	2500	1.4070	1.4030	28.14	43.6	43.5	0.001815	0.001810

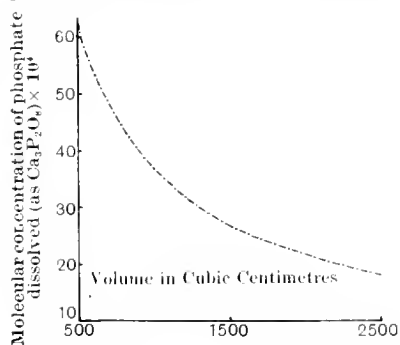


Diagram 1. See columns 8 and 9, Table III.

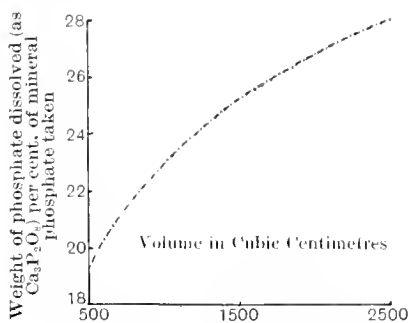
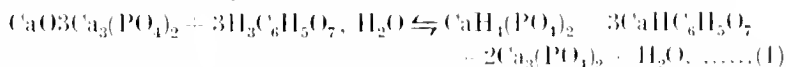


Diagram 2. See column 5, Table III.

It is seen from these results that the higher the dilution of citric acid, with m_1 m_2 kept constant, the greater is the proportion of citric solubility of the phosphate expressed as a percentage of the weight of the sample (column 5, Table III). Bassett¹ considers that the compound usually present in mineral phosphates is hydroxyapatite which may be written $[\text{Ca}_3(\text{PO}_4)_2]_3\text{Ca}(\text{OH})_2$. He also thinks it probable that hydroxyapatite is the only calcium phosphate that can permanently exist under normal soil conditions. It forms the stable solid phase over a range of acidity of great practical importance, as it can exist in contact with faintly acid, neutral or alkaline solutions. An attempt has been made to fit a theoretical curve to this series of experiments on the assumptions that there is equilibrium at the end of the experiment and that the following equation represents the reaction:



Of course other equilibrium equations including dicalcium phosphate can be written from which the same mass action equation can be deduced. The above equation is merely given as a suggestion.

The sample of mineral phosphate contained a proportion of CO_2 equivalent to 0.65 gram of calcium carbonate in 5 grams of the sample. Hence 1.36 grams of citric acid would be used up in the formation of citrates, leaving 8.64 grams of acid available to attack the phosphatic compound. If the original acid concentration is taken to be proportional² to the concentration at equilibrium, a constant should be obtained on applying the law of mass action. A good agreement between theory and observation is obtained on this hypothesis. If w = molecular concentration of acid after alkaline lime has been neutralised; u = molecular concentration of phosphate (expressed as tricalcium phosphate) at the end of 30 minutes agitation then we should have $u^4/w^3 = k$. The last two columns (columns 8 and 9, Table III) show the observed molecular concentrations and the theoretical values on the basis of above equation. A good fit is also obtained using the equation $u^4/(w-u)^2(w-2u)$ when the theoretical values are found by Horner's method. Diagrams 1 and 2 show in graphical form the results of Table III.

¹ *Trans. Chem. Soc.* 1917, **111**.

² The values of u from the equation $u^4(w-3u)^3$ show greater divergences from observational values than the values obtained from either of the equations given in the text. The ratio $\frac{(w-3u)}{w}$ varies from .72 to .80, showing that the original concentration is nearly proportional to concentration at the end of 30 minutes shaking. Whatever the reason, the formula u^4/w^3 gives by far the best fit to the results.

A second set of experiments was carried out in which the ratio $m_1/m_2 = 10$ instead of 2, that is, in each case 10 times more citric acid than phosphate was used in each separate experiment. The undernoted table (Table IV) shows the quantities used and the results obtained. This set of experiments when plotted against the theoretical curve expressed in equation (1) was found to be a very bad fit. In order to determine the best fitting equation the ratio of the exponent x to the exponent z in the general equation $C = \frac{k_1 u^x}{k_2 w^z}$ was determined where C , k_1 and k_2 are constants. The ratio $\frac{\text{mean } x}{\text{mean } z}$ was found to be equal to $1.0669 = 16/15$.

The following table (Table IV) and accompanying diagrams (Diagrams 3 and 4) show the observed results and those reached from the equation,

$$C = \frac{u^{16}}{w^{15}}, \text{ or } \log C = 16 \log u - 15 \log w.$$

Table IV. *Quantities of mineral phosphate and citric acid constant—dilution varied.*

(10 grams citric acid
1 gram mineral phosphate)

1 Exp.	2 Vol. of solution	3 4 Weight of phosphate dissolved as $\text{Ca}_3\text{P}_2\text{O}_8$		5 Percent dissolved of total $\text{Ca}_3\text{P}_2\text{O}_8$ content	6 Phosphate dissolved per cent. of weight of sample	7 8 Molecular concentration at end of 30 minutes	
		Observed	Theory			Observed	Theory
1	500	0.5376	0.5388	83.3	53.76	0.003468	0.003476
2	625	0.5472	0.5464	84.8	54.72	0.002824	0.002820
3	833	0.5610	0.5565	87.0	56.10	0.002172	0.002154
4	1250	0.5708	0.5705	88.5	57.08	0.001472	0.001473
5	2500	0.5955	0.5960	92.3	59.55	0.000768	0.000769
6	5000	0.6180	0.6220	95.8	61.80	0.000399	0.000401

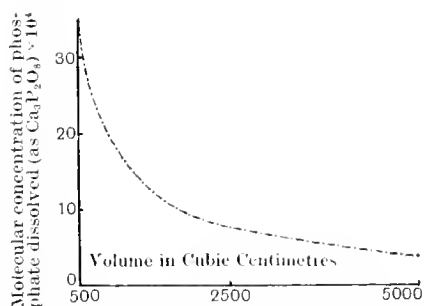


Diagram 3. See columns 7 and 8, Table IV.

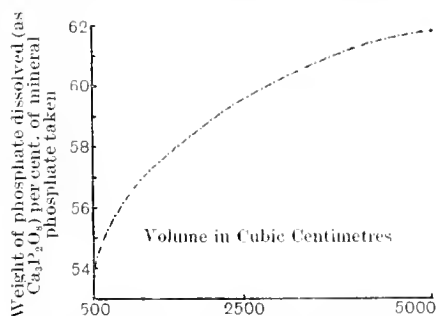


Diagram 4. See column 6, Table IV.

Experiment 6 of Table IV is an experiment identical in character with the private test already mentioned (see Table I). The results of experiments parallel to this experiment were used by certain sellers to describe the citric solubility of the mineral phosphate put on the market. It will be seen that Exp. 6 falls naturally into its place among the experiments given in Table IV. The amount of phosphate dissolved amounts to 62.2 per cent. of the weight of mineral phosphate tested while the same phosphate, analysed in accordance with the official test (Table III, Exp. 1) shows a citric solubility of 19.24 per cent. If we consider the proportion dissolved in relation to the total amount of phosphate present expressed as tricalcic phosphate it is found that, by the official method 29.83 per cent. of the total $\text{Ca}_3\text{P}_2\text{O}_8$ content is dissolved, while by the private test (at great dilutions, see Table IV) 95.8 per cent. of the total $\text{Ca}_3\text{P}_2\text{O}_8$ content was dissolved. These results show what might naturally be expected, namely, a much higher solubility of mineral phosphate in the experiments where the constant ratio $m_1/m_2 = 10$ (Table IV) was used than where $m_1/m_2 = 2$ (Table III) was used. In other words if m_1/m_2 is made large enough we should reach the limit of 100 per cent. citric solubility for all very high dilutions.

The results in Table II show that, using the official test on both slags and mineral phosphates, slags generally show a higher citric solubility than mineral phosphates. If the unofficial test (Exp. 6, Table IV) was universally applied to slags we should have similar high solubility figures, in other words the citric solubility at high dilution would be practically 100 per cent. and the only item of information which would be valuable to the purchaser would be the actual proportion of phosphate, expressed as tricalcium phosphate, present in the fertiliser. In some slags the citric solubility in terms of total phosphate content is as much as 90 per cent. (see Table I) and therefore the actual increase on dilution must be necessarily small compared with the increase in citric solubility on dilution of any mineral phosphate.

The undernoted table (Table V) shows the average composition of five commercial mineral phosphates¹. The sixth (Egyptian) was analysed in my laboratory.

Since calcium carbonate is present in varying proportions in commercial mineral phosphate it is clear that with a constant initial molecular concentration of citric acid, varying quantities of citric acid will be available to attack the insoluble phosphate. For example, suppose we selected two different varieties of mineral phosphate, ground to the same

¹ Robertson, *J.S.C.I.*, **35**, p. 218.

degree of fineness, and containing the same proportions of hydroxy-apatite but quite different proportions of calcium carbonate. The sample which contained the smaller quantity of calcium carbonate would show a higher citric solubility than the second sample which contained a higher proportion of calcium carbonate, due to the presence of a relatively large proportion of free citric acid in the former.

Table V. *Composition of Mineral Phosphates.*

		Makatea Island	Florida Pebble	Algerian	Gafsa	Tunisian	Egyptian
Calcium oxide	52.38	47.10	46.13	43.30	48.40	46.81
Phosphoric acid	38.24	31.50	27.27	25.35	26.13	29.52
Carbon dioxide	1.69	3.04	6.70	5.50	9.03	9.42
Moisture	1.46	1.00	0.72	3.26	0.98	—
Combined moisture and organic matter ...	}	3.39	2.41	3.28	4.39	3.21	2.06
Ferric and aluminium oxides ...	}	1.01	1.60	2.59	4.98	4.25	2.78
Magnesium oxide	1.35	1.90	2.02	1.72	0.90	0.77
Sand	0.28	7.03	8.12	7.56	4.65	7.73
Undetermined	0.20	4.42	3.17	3.94	2.45	0.91

Citric solubility is not necessarily a test of the availability of the phosphate to the plant in the soil. If the sample is finely ground and has a low citric solubility, the lowness of the citric solubility in the case of mineral phosphate would mainly be due (1) to the presence of alkaline material which would neutralise a large proportion of the citric acid, leaving the residue to act on the phosphate and (2) to the chemical constitution of the phosphatic mineral. In the case of slags the citric solubility would be mainly dependent on (1) the compounds of fluorine as shown by Robertson, (2) the presence of alkaline lime as shown by Ramsay, and (3) the chemical constitution of the phosphatic compound in the slag. It has yet to be shown that the phosphate in mineral phosphate is not utilised by the plant as readily and as efficiently as the phosphate from slags. In other words the exact chemical composition of the phosphatic compounds in the various mineral phosphates and slags has, in each case, to be demonstrated. It appears to be necessary to test the citric solubility of phosphates of *known composition* against their availability in the soil as shown by yield of crop. The results would then show how far, if at all, citric solubility is a measure of availability in the soil. Since the commercial fertilisers tested contain varying quantities of alkaline lime, fluorides and other interfering substances, and since the chemical constitution of the fertilisers is incompletely known, the writer can see no scientific validity in the use of citric solubility as a measure of availability. The three practical tests appear to be:

- (1) Total phosphatic content.
- (2) Degree of fineness of the powder.
- (3) Presence or absence of substances capable of inhibiting growth.

A fourth test of scientific, as well as practical, value would be a test demonstrating the constitution of the phosphatic compounds in the fertiliser.

If citric solubility cannot be regarded as a useful and practical test for mineral phosphate it is still less valid as a comparative test for slags and mineral phosphates alike owing to the varying composition of slags and to their widely different chemical composition and constitution when compared with mineral phosphates.

In regard to the chemical constitution of phosphatic fertilisers it has already been noted that hydroxyapatite is considered by Bassett as the chemical compound probably present in mineral phosphate. Morison states¹ that the molecular ratio of phosphoric anhydride to calcium oxide P_2O_5 CaO is 1.5 in slags and supports Stead's conclusions that the phosphatic content in basic slags consists of a chemical union of tetra-calcium phosphate and monocalcium silicate $(CaO)_4P_2O_5 \cdot CaOSiO_2$. On the other hand the ratio of phosphoric anhydride to calcium oxide in Bassett's hydroxyapatite is $3.10 = 1.3\frac{1}{2}$. Morison also deals with the effect of free lime on the citric solubility of slags and shows that the greater the amount of free lime in a slag the greater is the total solubility after three extractions. On the other hand Ramsay² shows that about 91 per cent. of the total phosphoric acid in pure tricalcium phosphate is soluble in the prescribed 2 per cent. citric acid solution. This degree of solubility is very similar to the degree of solubility of the best grades of slags (see Table I). He also shows that by the simple addition of calcium carbonate to pure tricalcium phosphate the citric solubility is reduced from 91 to 84 per cent. This is naturally to be expected and the apparent greater solubility of phosphate with increase of lime content found by Morison must be due to other causes. It should be noted that the quantities of free lime present in Morison's samples are relatively small. The increase in solubility with an increase of silica and the decrease in solubility in the presence of fluorides have already been mentioned. Robertson finds that calcium carbonate decreases substantially the solubility of phosphates as judged by the 2 per cent. citric acid test. The exact effect of the presence of calcium carbonate or calcium hydroxide on the solubility of phosphate of a known composition can of course be found

¹ *Journ. Agri. Sci.* 1909.

² *Ibid.* 8, p. 277.

a priori. The difficulty arises when the composition of the phosphates is unknown and when other interfering substances are present. Since the nature of the substances present are unknown, equations expressing the law of mass action cannot be written down in these cases.

The following table (Table VI) shows the average composition of some commercial slags as given by Collins (*Chemical Fertilisers*, p. 122).

Table VI. *Composition of Slags.*

			1	2	3	4	5
Total P_2O_5	12.60	20.49	9.09	17.57	19.35
Silica	17.69	10.12	13.49	7.77	12.12
Lime	38.02	46.81	40.43	52.22	44.75
Magnesia	4.24	2.92	5.01	1.94	0.11
Manganese oxide	7.39	4.38	5.41	9.37	4.68
Iron	12.89	9.98	13.83	8.13	9.10

These latter tables (Tables V and VI) show in a general way the differences between slags and mineral phosphates. These two classes of fertilisers contain non-phosphatic residues differing in chemical composition, and residues which are common to both in different proportions. The results of this section show that citric solubility is merely a special case of the law which has been proved to hold for the solubility of a definite chemical substance in dilute acids and it can always be stated *a priori* when the conditions are known for a definite substance in a definite dilution. When, however, we pass from a single substance to mixtures of varying composition citric solubility cannot be descriptive of available phosphate of definite composition. The reason for this lies (1) in the unknown changes which take place in the initial molecular concentration of the citric acid, due to the formation of calcium and other citrates from the carbonates and hydrates present in the fertiliser, (2) in the unknown changes which take place on agitating phosphatic fertilisers of varying composition and (3) in the known effects produced by the presence of fluorides and of silica.

V. EXPERIMENTS IN WHICH THE VOLUME OF FLUID AND THE WEIGHT OF MINERAL PHOSPHATE ARE BOTH CONSTANT, THE VARYING FACTOR BEING ACID CONCENTRATION.

We shall now consider condition (2) namely, where the amount of mineral phosphate (m_2) and the volume of fluid (m_3) are both constant, i.e. $m_3/m_2 = \text{constant}$, while the amount of citric acid is varied.

The following series of experiments was carried out with a constant weight of mineral phosphate (5 grams) in varying concentrations of citric acid in a constant volume of 500 c.c. The undernoted results (Table VII)

show that if the acidity is expressed as the molecular concentration of citric acid at the end of 30 minutes shaking, $K = n^4/n^3$ fairly accurately describes the solubility of the phosphates at the acid concentrations named.

Table VII. *Amount of mineral phosphate present and volume constant — acidity varies.*

m_3/m_2 100; vol. 500 c.c. m_3/m_2 mineral phosphate 5 grams								
Exp	Wt. citric acid in 500 c.c.	Acidity as citric acid at end of 30 minutes	Wt. of phosphate dissolved as $\text{Ca}_3\text{P}_2\text{O}_8$ in 500 c.c.		Per centage dissolved of total $\text{Ca}_3\text{P}_2\text{O}_8$ content	$\text{Ca}_3\text{P}_2\text{O}_8$ found per cent of phosphate taken	Molecular concentration of phosphate at end of 30 minutes	
			Observed	Theory			Observed	Theory
1	10 grms.	8.87 grms.	0.9675	0.9492	30.0	19.35	-006242	-006124
2	8 ..	7.02 ..	0.8119	0.7958	25.2	16.24	-005238	-005134
3	6 ..	5.11 ..	0.6743	0.6250	20.9	13.48	-004350	-004032
4	4 ..	3.25 ..	0.4495	0.4470	13.9	8.99	-002900	-002884
5	2 ..	1.34 ..	0.2176	0.2300	6.7	4.35	-001404	-001484
6	1 ..	0.49 ..	0.1020	0.1082	3.2	2.04	-000658	-000698

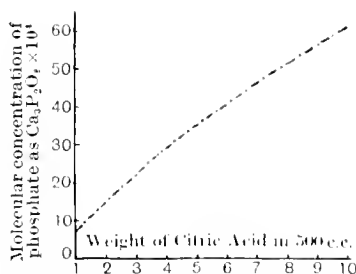


Diagram 5. See columns 8 and 9, Table VII.

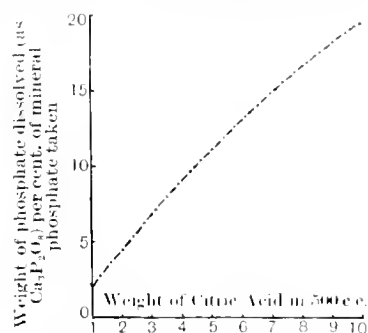


Diagram 6. See column 7, Table VII.

The accompanying diagrams (Diagrams 5 and 6) show the results of Table VII. These results show clearly increasing citric solubility with increasing acid concentration but with constant weight of mineral phosphate in a constant volume. In a citric solubility test, used as a measure of availability, it seems necessary therefore to show that a two per cent. solution is the concentration of citric acid best suited for the utilisation of phosphate by the plant. Would it not be more in accordance with scientific practice firstly, to ascertain the constitution of the phosphatic fertilisers, and secondly, to determine what rôle concentration has in the life history of the plant?

VI. EXPERIMENTS WITH CONSTANT VOLUME AND CONSTANT CONCENTRATION OF ACID BUT WITH VARYING QUANTITIES OF MINERAL PHOSPHATES.

In the first set of experiments fairly high constant values of m_1/m_2 were used. We shall now consider the effect of making m_1/m_2 small, m_1 and m_3 being in this case constant and m_2 the variable. If m_1/m_2 is made small enough we should have a citric solubility practically zero for all dilutions. An example indicating the approach to the latter condition is given in the following table (Table VIII) where the third set of conditions is observed. (See also Diagrams 7 and 8.)

Table VIII. *Amount of citric acid and volume constant—amount of mineral phosphate (m_2) used varied.*

		$m_3 = 50$		{ 10 grams citric acid = m_1 1500 c.c. volume = m_3		
Exp	Wt of mineral phosphate taken = m_2	Ratio m_1/m_2	Acidity expressed as citric acid at end of 30 minutes	Amount of phosphate dissolved as $\text{Ca}_3\text{P}_2\text{O}_8$	Mol. conc of phosphate dissolved at end of 30 minutes	Citric solubility expressed as $\text{Ca}_3\text{P}_2\text{O}_8$ per cent of phosphate taken
1	5 grams	2	8.87	0.9705	.006261	19.41
2	10 "	1	8.50	0.8660	.005587	8.66
3	20 "	0.5	7.65	0.6070	.003916	3.04
4	40 "	0.25	5.98	0.4110	.002652	1.03

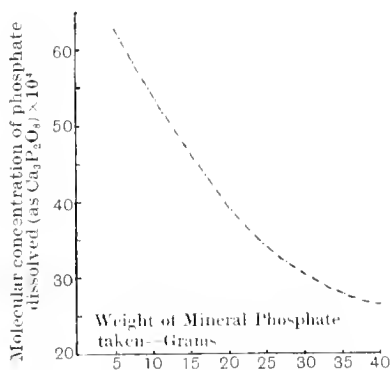


Diagram 7. See Table VIII.

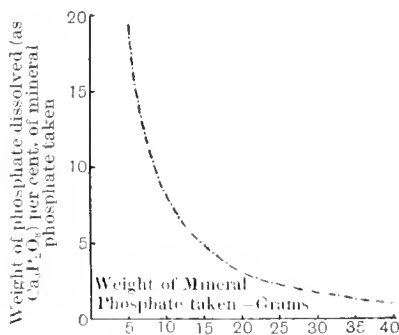


Diagram 8. See Table VIII.

If the mineral phosphate contained merely tri- or di-calcium phosphate and was quite free from $\text{Ca}(\text{OH})_2$, CaO or CaCO_3 and also was in excess, mere variations in the quantity taken would have had little or no effect on the amount of citric acid present at the end of the period of shaking. The presence of $\text{Ca}(\text{OH})_2$ naturally reduces the acid concentration with the result that, while 19.41 per cent. of the 5 grams mineral phosphate was dissolved only 1.03 per cent. mineral phosphate was dis-

solved when 40 grams of the fertiliser were taken. It is thus shown, which is also apparent *a priori*, that citric solubility depends on the quantity of mineral phosphate or slag used. With quantities like 50 grams or 100 grams of mineral phosphate the citric solubility would be extremely small. On what theoretical grounds are quantities like 5 grams (official test) in the case of slag and 1 gram (private test) in the case of mineral phosphate used to determine the availability of phosphate in the soil?

VII. THE INCOMPLETENESS OF THE REACTIONS.

All the foregoing results have been considered from the standpoint of the amount of phosphate dissolved per cent. of the weight of mineral phosphate taken. It is desirable, however, to consider the amount of phosphate dissolved in relation to the amount theoretically obtainable if the reaction were complete. We cannot say definitely what the reaction is but we know that the equation in Section IV gives a suitable prediction formula. Suppose we put the question: What proportion of the amount indicated by this equation is obtained in each separate experiment? We know from theory that the hydrogen ion concentration of an acid is increased by dilution and we should therefore expect *greater proportions of the possible total amounts* at higher dilutions than at lower dilutions. Let us consider the first series of experiments from this standpoint. If the citric acid concentration at the beginning of the reaction was $3w$ gram-molecules and the reaction went completely, according to the equation given in Section IV we should have w gram-molecules of monocalcium phosphate formed, equivalent to w gram-molecules of tricalcium phosphate. Hence for the completed reaction we should have w gram-molecules of citric acid giving $1/3 w$ gram-molecules of tricalcium phosphate. Suppose we find only y gram-molecules of tricalcium phosphate then $\frac{3y}{w} \times 100$ is the percentage of the theoretical amount which has been found. The following table (Table IX) shows the concentration of the citric acid, the theoretical amount of phosphate obtainable in a complete reaction according to the equation, the actual amount of phosphate obtained, as gram-molecules of tricalcium phosphate per litre, and the percentage of the theoretical amount of phosphate obtainable.

It is seen that this percentage is practically identical with the percentage in col. 5 of Table III. Indeed the numbers in col. 5 of that table multiplied by 1.016 give the percentage in col. 4 of Table IX. This arises from the following considerations. If a_1 be the weight of phosphate

dissolved and a_2 = weight of mineral phosphate taken for the particular dilution v then $100 \frac{a_1}{a_2} = p_1$ = amount of phosphate dissolved as a percentage of amount of mineral phosphate taken. The number of gram-molecules of citric acid per litre in each case may be written $\frac{10}{210} \cdot \frac{1000}{v} = w$ where v is the dilution. The amount of phosphate theoretically obtainable expressed as tricalcium phosphate is therefore $w/3 = x$ gram-molecules per litre. The amount of phosphate found also expressed as tricalcium phosphate in gram-molecules per litre is $\frac{a_1}{310} \cdot \frac{1000}{v} = y$.

Table IX. *Table showing amount of phosphate found per cent. of amount theoretically obtainable, at various dilutions on basis of equation, Section IV.*

Exp.	Gram-molecules per litre			Phosphate found per cent. of amount theoretically obtainable
	Concentration of citric acid	Phosphate theoretically obtainable in complete reactions	Phosphate actually obtained	
1	·0952	·0317	·0186	19·55
2	·0762	·0254	·0158	20·75
3	·0572	·0191	·0126	22·10
4	·0381	·0127	·0094	24·55
5	·0190	·0063	·0054	28·59

Now the number of gram-molecules dissolved expressed as a percentage of the number of gram-molecules theoretically obtainable is clearly

$$\frac{y}{x} \cdot 100 = p_1 \times \frac{63a_2}{310} = p_2.$$

In the series under consideration $a_2 = 5$ and thus we have

$$p_2 = p_1 \cdot \frac{63}{62} = 1·016 p_1.$$

With increasing values of a_2 it is known that a smaller series of values of p_1 would be obtained (see Table VIII). For example, if 40 grams of mineral phosphate were taken then $p_2 = 8p_1$ (approximately). The results in Table IX merely illustrate the well-known fact that solubility depends on the hydrogen ion concentration. They further show that it is misleading to adopt a particular set of weights to test citric solubility and to express the solubility in terms of the weight of mineral phosphate taken for the experiment, if it is intended to judge the quality of a phosphatic fertiliser by the result so obtained.

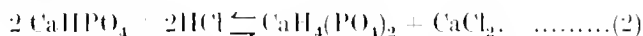
VIII. DICALCIUM PHOSPHATE EXPERIMENTS.

The observed amount of phosphate dissolved by citric acid per cent. of the theoretical amount for a completed reaction found in these experiments may be contrasted with corresponding figures obtained from the interaction between a pure substance like dicalcium phosphate and hydrochloric acid. A series of experiments was conducted with this end in view. In each experiment a constant quantity (25 grams) of precipitated dicalcium phosphate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) was shaken for half an hour in a constant volume (500 c.c.) of water. The proportion of hydrochloric acid was varied as shown in Table X. The following results were obtained:

Table X.

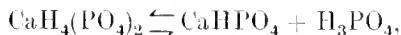
Volume constant = 500 c.c.; dicalcium phosphate = 25 grams					
	I	II	III	IV	V
	Original concentration of HCl Gram mols. per litre	Concentration of phosphate after shaking (gram mols. $\text{CaH}_4(\text{PO}_4)_2$ per litre) = u	Theoretical concentration of phosphate $\frac{k}{1+2k} \cdot a$	Grams of dicalcium phosphate dissolved per litre	
Exp.	a			Observed	Theory
1	·2856	·1299	·1325	44·69	45·58
2	·2285	·1058	·1060	36·40	36·16
3	·1713	·0792	·0795	27·24	27·35
4	·1142	·0517	·0530	18·82	18·23
5	·0571	·0265	·0265	9·12	9·12
6	·0286	·0124	·0133	4·27	4·58

These results show the decreasing percentage of phosphate dissolved with decreasing acid concentration as usually obtained in such experiments. The result, however, to be contrasted with the corresponding previous results, is the relative completeness of the reaction as seen in cols. I and II of the above table (Table X). Since there is excess of dicalcium phosphate the undernoted equation may be taken to represent the reaction namely,



In this equation two molecules of hydrochloric acid are used up to produce one molecule of monocalcium phosphate and one molecule of calcium chloride. Hence if we start with w gram-molecules of hydrochloric acid we shall have, for a completed reaction, $\frac{1}{2}w$ gram-molecules of monocalcium phosphate, the substance determined. If $w =$ molecular concentration of hydrochloric acid per litre at the beginning of the experiment and $u =$ molecular concentration of phosphate expressed as $\text{Ca}_3(\text{PO}_4)_2$ per litre at the end of 30 minutes shaking when equilibrium may be presumed in this case to be established, then according to the

law of mass action we should expect $k = u/w - 2u$ to describe the experimental results. An inspection of col. III (Table X) will show that the formula reasonably fits the data. No account has been taken of the degree of ionisation in the above equilibrium equation. The extent of the reverse action,



has also been neglected.

If the degree of ionisation of hydrochloric acid is taken into account together with the fact that a minute quantity of calcium hydroxide was found to be present in the dicalcium phosphate used it is found that $2u$ is slightly greater than w as may be seen on inspection of Table XI (col. IV). The value of $2u/w$ varies from 1.008 to 1.039, the average value being 1.0228 and theory requires this ratio to be equal to unity for a completed reaction.

Table XI.

I	II	III	IV
Concentration of dissociated HCl (gram-mols. per litre)	Gram-mols. $\text{CaH}_4(\text{PO}_4)_2$		
$=w$	$=u$	$2u$	$\frac{2u}{w}$
.2576	.1299	.2598	1.0085
.2087	.1058	.2116	1.0139
.1573	.0792	.1584	1.0070
.1053	.0547	.1094	1.0389
.0514	.0265	.0530	1.0311
.0239	.0124	.0248	1.0377

The work in this section is intended merely to illustrate the approximation to a completed reaction in the case of a known phosphate. More detailed work is necessary and a fuller consideration of the theory is also necessary in order to give a complete physico-chemical explanation of the phenomena.

IX. CONCLUSIONS.

(1) If constant weights of sample and of citric acid are used in a series of experiments, the only quantity varied being the volume, the quantity of phosphate dissolved per cent. of weight of sample taken increases with increasing volumes. With 5 grams of sample and 10 grams of citric acid, the citric solubility varied from 19.24 to 28.14 per cent. of weight of sample. With 1 gram of sample and 10 grams of citric acid, the citric solubility varied with increasing dilution from 53.8 to 61.8 per cent. of weight of sample. The effect of the presence of alkaline lime was eliminated by maintaining m_1/m_2 at a constant value and with increasing dissociation as a result of increasing dilution increasing percentages of

phosphate were dissolved (i.e., percentages of mineral phosphate taken). The molecular concentration on the other hand (i.e. number of gram-molecules per litre) decreased with increasing dilution. It is not held that the equations describing the results are valid for concentrations or dilutions outside those used in these experiments.

(2) If a constant weight of sample and a constant volume are used in a series of experiments, the concentration of citric acid being varied from 1 gram to 10 grams, the citric solubilities varied from 4.1 to 19.1 per cent. of weight of sample taken. That is to say, citric solubility increased, as we should expect, with increased acid concentration at constant volume and with a constant weight of hydroxyapatite.

(3) If a constant weight of citric acid and a constant volume are used in a series of experiments, the quantities of sample being varied from experiment to experiment, the citric solubilities decreased from 19 to 1 per cent. of weight of sample taken. These decreases are due (1) to the presence of $\text{Ca}(\text{OH})_2$ in the molecule of hydroxyapatite, (2) to the presence of $\text{Ca}(\text{OH})_2$, CaO or CaCO_3 in the free condition and (3) to the fact that the results are expressed in terms of mineral phosphate taken for analysis.

(4) It is seen that, other conditions being constant, citric solubility depends upon an unlimited choice of constant values of any two factors, together with an unlimited number of values of the third varying factor. If, therefore, a citric solubility test has to be adopted, the theoretical condition determining the relative quantities of sample, acid and volume must be found, otherwise the test has no practical value. Wagner has not supplied any theoretical basis for selecting the specified constant quantities of the three factors. We can, therefore, at will select for slags suitable values of sample, acid and volume to secure high citric solubility values. We can, however, select at will quite different values of sample, acid and volume which will give equally high citric solubility values for mineral phosphates. Further, we could select for both slags and mineral phosphates suitable values of sample, acid and volume which would give, on the one hand, perfect citric solubility (100 per cent.) or, on the other hand, no citric solubility at all.

(5) The citric solubility of mineral phosphate has been contrasted with the solubility of a pure substance, dicalcium phosphate, in hydrochloric acid. It is shown that with the dilutions used and with an excess of dicalcium phosphate the reaction is practically complete. Since there is an excess of dicalcium phosphate it has been assumed that the main substance present at the end of the reaction is monocalcium phosphate.

(6) Citric solubility, if applied to fertilisers may in a certain degree

be a measure (1) of fineness of grinding as already pointed out by other workers, but it seems necessary also to postulate similarity of composition in comparing degrees of fineness in practice, (2) of the presence or absence of alkaline substances in fertilisers approximately of the same composition and ground to the same degree of fineness, (3) of the presence or absence of fluorides as well as alkaline substances in slags and (4) of the differences in the constitution of the phosphatic compounds in finely ground fertilisers containing approximately the same proportions of extraneous substances.

None of these conditions are realisable in practice because the proportions and the actions of extraneous substances are generally undetermined and because the nature of the phosphatic compound present is either unknown or is not given. Further we can with any phosphatic fertiliser irrespective of its composition and constitution, use concentrations or dilutions to get any value of citric solubility we please. Citric solubility is therefore an unreliable empirical test of the agricultural value of mineral phosphates and slags. It has yet to be shown that high citric solubility is a measure of the presence of phosphate in a readily available condition for plant growth, or is an indication of the presence of a highly citric soluble phosphatic compound of a known chemical constitution. We are driven, therefore, to the conclusion that the only practical tests of value of phosphatic fertilisers from the agricultural standpoint are:

- (1) Total phosphatic content.
- (2) Degree of fineness of grinding.
- (3) Freedom from injurious substances and of substances inhibiting plant growth.

The effect of using different kinds of phosphatic fertilisers on yield of turnip crop, where the same amount of fertilisers, expressed as tricalcium phosphate, is applied in each case forms the subject of a separate communication.

(Received December 23rd, 1921.)

COMPARATIVE DETERMINATIONS OF THE DIGESTIBILITY AND METABOLISABLE ENERGY OF GREEN OATS AND TARES, OAT AND TARE HAY AND OAT AND TARE SILAGE.

BY HERBERT ERNEST WOODMAN, PH.D., D.Sc.

(*From the Institute for the Study of Animal Nutrition,
School of Agriculture, Cambridge University.*)

IN a recent communication¹, the results of an investigation into the digestibility of oat and tare silage were recorded. The desirability, however, of extending the scope of this initial work was recognised, in view of attempts which are being made to re-establish on a large scale the practice of ensilage in this country.

Before incurring the initial expense involved in the setting up of a silo, the farmer is justly entitled to ask and receive answers to such questions as the following:

(1) What are the precise conditions under which the production of a palatable silage of good quality can be guaranteed?

(2) What is the magnitude of the losses of nutrient matter sustained by the crop when stored in the silo? Are such losses greater or smaller than those which accompany storage in the haystack?

(3) Does the green forage suffer any marked diminution in digestibility and nutritive value during its conversion into silage, and what are the relative merits of the silo and the haystack in this respect?

(4) What are the best kinds of forage to be grown in this country for the purposes of ensilage?

In America, where the practice of ensilage has been well established on a very large scale for many years, numerous trials have been carried out at the various Experiment Stations with a view to throwing light on these questions. The conditions obtaining in that country, however, are markedly different from English conditions. The maize plant is the forage which is widely grown in America for the production of silage, and it has been found to be in every respect excellently adapted to this purpose. In this country, however, little success has so far attended the efforts to

¹ Wood and Woodman, *Journ. of Agric. Sci.*, **11**, 304, 1921.

grow and utilise maize forage for ensilage, and consequently the attention of agriculturists has been directed towards the discovering of other crops which are suitable for being grown for silage. A large measure of success has attended these efforts. Amos¹, for instance, has shown that a mixed crop of oats and tares possesses all the characteristics for the successful inclusion of the practice of ensilage in the ordinary farm routine.

It would, therefore, be unsafe to assume that the results obtained in American trials with maize forage apply with equal force to the methods of ensilage which are being adopted in this country. Concurrently with the extension of the practice, it is necessary to prosecute enquiries with a view to obtaining satisfactory answers to such questions as those enumerated above. Investigations are proceeding in all these directions at Cambridge, and the work to be detailed in the present communication was undertaken in order to obtain a direct comparison of the digestibility of oat and tare silage not only with oat and tare hay, but also with the green oats and tares from which both hay and silage had been produced. Such information must be taken into account when coming to a decision as to the relative merits of ensiling and hay making.

From prior considerations, it would be natural to presume that the processes of hay and silage making would result in a diminution of the digestibility of the ingredients of the green forage, since it seems reasonable to assume that whatever fermentative or bacterial changes take place, do so mainly at the expense of the more readily assimilated constituents. The characteristic change which occurs when green forage is packed into the silo involves the destruction of carbohydrates with the formation of organic acids, like lactic and acetic acids. This is the result of fermentation mainly brought about by the enzymes of the plant cells and by bacteria. Since the more easily assimilated carbohydrates are liable to be used up in this process, it would be anticipated that the residual carbohydrate ingredient of the silage would have a diminished digestibility. On the other hand, the percentage of digestible ether extractable material in the forage will be augmented by the formation of the organic acids, although the net result of the change must involve loss of nutritive value. This loss, however, need not necessarily, on theoretical grounds, be considerable, since these changes are quickly arrested after the development of a sufficient degree of acidity. In any case, it should be remembered that all types of roughage suffer similar destruction of soluble carbohydrates during the time they stagnate in the rumen of the animal.

¹ Amos, *Journ. of the Farmers' Club*, Part 2, 1920.

Similar considerations arise when forage is dried in the field and stored in the stack. If this could be carried out without undue fermentation taking place, then there seems no particular reason why the mere drying down should result in any appreciable depression of the digestibility. In the usual practice of hay making, however, field fermentation and heating in the stack tend to deprive the forage of quite considerable amounts of its more easily assimilated organic matter, and the usual effect is to cause a decrease in the digestibility of the protein and the nitrogen-free extractives.

The possibility must not be lost sight of that the changes which occur in the silo and the haystack may be accompanied by an actual increase in the digestibility of the less easily assimilated ingredients of the green forage. For instance, evidence is not lacking that the heating which occurs both in the silo and the haystack leads to an increase in the digestibility of the crude fibre constituent.

The changes which affect the protein ingredient of ensiled forage, resulting in an increase in the amount of amino acids, may be expected to lead to an increase in the "ease" of digestibility of the material, rather than to an increase in the actual protein digestion coefficient, since the same type of change is readily brought about in the digestive tract of the animal. The palatable and succulent nature of good silage should give it a distinct advantage over the dried fodder in regard to the ease with which the animal is able to digest it. Like other succulent foods, silage is reputed to have a beneficial effect on the digestive organs, although, of course, a poor quality of silage possessing undue acidity may have the reverse effect.

The available data in connection with digestibility trials on corn forage agree substantially with the foregoing considerations. The following table gives the average digestion coefficients obtained in a large number of American trials for corn silage and green and cured corn fodder¹.

Forage	Dry matter	Ash	Protein	Crude fibre	N-free extractives	Ether extract
Green corn fodder, %	68	35	61	61	74	74
Cured corn fodder, %	66	34	55	66	69	72
Corn silage, %	66	31	53	67	70	81

These figures afford some idea of the relative digestibilities of the different ingredients of the three types of fodder, although it is not certain that in every case the results were obtained by strictly comparable trials. It will be noted that there is no appreciable difference in the

¹ Henry, *Feeds and Feeding*, 1902, p. 248.

digestibility of the corn silage and dry corn fodder, the most marked difference being in the case of the ether extract, as would be anticipated. Both stored fodders are somewhat less digestible than the green fodder, although it is of interest to note that in the case of the crude fibre, the percentage digestibility shows an increase in the case of both the silage and the dried corn fodder. It is also noteworthy that drying and ensiling do not appear to depress the digestibility of the nitrogen-free extract to any marked extent.

GENERAL ARRANGEMENT OF EXPERIMENT.

For the purpose of the digestion trials, a plot of about 1600 square yards was measured off from a large field of oats and tares situated on the Howe Hill Experimental Farm at Cambridge. Within the limits of the experimental plot, the growth of the crop was, to the eye, of a reasonably uniform character.

In order to bring out in the sharpest manner possible any differences in the digestibilities of the three types of foodstuff, it was decided that the experiment should consist of three main periods in which ample rations of the green forage, hay and silage, unmixed with concentrates, should be fed successively and their respective digestibilities determined directly. In the earlier work on oat and tare digestibility, doubt was felt as to the desirability of keeping the sheep for a period of three weeks on an acidic food like silage alone, and the possibility of digestive disturbance was avoided by feeding a smaller ration of silage together with a basal ration consisting of meadow hay and a little linseed cake. This procedure necessitated the carrying out of digestibility measurements on the basal ration and the calculation of the silage digestibility by difference. In this experiment, however, no difficulty was encountered in maintaining the sheep during the three weeks of experiment on an ample ration of silage alone. The quality of the silage was good and the sheep from the outset consumed the ration quite readily without suffering the slightest discomfort. Indeed, it was noteworthy that less difficulty was experienced with the feeding during the silage period than in the green fodder and hay periods. When the green fodder was introduced into the diet, one of the sheep showed at first a tendency to be slightly "blown," but by judiciously cutting down the amount fed for some days, this difficulty was overcome and a very satisfactory trial was obtained. In the hay period, one of the sheep displayed an inability to consume the entire ration, and consequently the digestibility of the hay was deter-

mined on a smaller ration than was previously designed. Reference will be made to this point again at a later stage.

It was thus possible, by avoiding basal rations, to measure the digestibility of the silage directly on a much larger quantity of material than could possibly have been fed in an indirect method of determination, and, moreover, the conditions obtaining throughout the whole trial were thus made comparable.

The green oats and tares period began on June 16, 1921. Samples of about 25 lbs. were cut from the plot daily by means of a sickle and were passed through the chaffing machine before being fed to the sheep. During the few hours over which it was necessary to store the material, it was spread out in a thin layer on a concrete floor to prevent "heating," which occurred fairly readily when the cut fodder was kept in bulk in bags. The samples for analysis were taken at the same time as the rations for the whole day were weighed out. Moisture determinations were carried out on the representative samples every two days during the analytical period, the dry matter from such determinations being utilised in the making up of the period composite sample. The gradual increase of dry matter in the maturing crop is illustrated by the following figures obtained during the fourteen days' experimental period:

Days	1-2	3-4	5-6	7-8	9-10	11-12	13-14
Dry matter, %	29.00	30.95	33.69	32.55	33.23	33.55	34.32

As the approach of the crop to maturity was probably accompanied by a gradual diminution in the digestibility of the forage, it was necessary, in order to secure a fair comparison with the hay and silage, that the parts of the plot reserved for the hay and silage should be cut halfway through the green oats and tares period. This was accordingly done and the material to be converted into silage was carted without delay, passed through the usual cutting machine and then packed tightly into a small experimental silo reserved for this purpose. Further particulars will be given when the details of the silage period are discussed; it is sufficient to note here that all the conditions for making the silage were satisfactory and material of an excellent quality was obtained when the silo was opened at a later date. The making of the hay was not attended with similar good fortune. The first two days during which the forage was drying in the field were beautifully fine and sunny, but a sudden thunder-storm shortly before the time for carting rendered the sample quite unfit for use in a comparative trial with the silage. It was therefore necessary to use another sample of oat and tare hay which had been carted before

the storm, but which had been cut at the same time as the forage on the experimental plot. It came from a neighbouring part of the field, but, as will be seen later, this circumstance detracted somewhat from the strictly comparative nature of the trials. The hay sample was finally packed tightly into a meadow haystack standing in a Dutch barn and a thick layer of straw was pressed compactly on top, so that the water vapour generated by the heating in the stack should pass into the straw and not condense in the upper portions of the hay, thus causing it to mould.

The experimental procedure in the trials was, in the main, identical with that adopted in the earlier work on oat and tare silage digestibility. The Halnan harness was again employed with success, and there can be little doubt that, from the point of view of convenience, safety and comfort for the sheep, it is distinctly superior to the orthodox "funnel and bag" harness.

The analytical period in all three cases consisted of fourteen days, this being preceded by a preliminary period of seven days. Bi-weekly composites of urine and faeces were made, nitrogen estimations being carried out on these samples. Determinations of dry matter were made on aliquot portions of the faeces samples, the dried residues being preserved in air-tight bottles to be utilised in the making up of the period composite samples for complete analysis. Separate period urine composites were kept for dry matter estimations and the determination of the energy content by means of the bomb calorimeter. They were preserved by the addition of chloroform.

In the hay period, the daily rations for the whole period were weighed out previously into paper bags from the bulk of chaffed hay, the samples for complete analysis and moisture determinations being drawn at the same time. In the silage period, as in the green forage period, moisture determinations were made on representative samples every two days, the dried material being made up into the composite samples for complete analysis. It was not considered necessary to carry out determinations of nitrogen on the *fresh* silage samples, since in the earlier work with oat and tare silage, it was noted that no measurable loss of nitrogen occurred during the drying down of silage.

In order to gain a trustworthy comparison of the digestion coefficients of the protein constituents of the three foodstuffs, it was essential to take into account the well-established fact that the faeces do not consist solely of undigested food residues, but that the latter are largely contaminated by nitrogenous metabolic products which have been

secreted into the alimentary tract and escaped re-absorption. Thus the protein digestion coefficients as determined directly represent minimum values, and in view of the possibility of this disturbing factor operating unequally in the trials with the different fodders, it was deemed advisable to correct the protein digestibilities by basing them on the amount of pepsin-insoluble nitrogen in the faeces. Accordingly, determinations of the pepsin-insoluble nitrogenous constituents of the faeces were made in all three cases. The error occasioned by the presence of metabolic products in the faeces affects, of course, the accuracy of the digestion coefficients of all the foodstuff ingredients, but it falls with especial weight upon the protein and ether extract. No satisfactory and reliable method for correcting the ether extract digestibility has so far been evolved¹.

The sheep were weighed at the beginning and end of each period and account was kept of the nitrogen balance. The wethers employed were the same as were used in the earlier work on oat and tare silage. A study of the results shows an extraordinarily good agreement between the sets of figures obtained for the two sheep. Such agreement is not often met with in work with animals, and it would appear that the two sheep selected for the purpose possessed almost equal digestive capacity.

The writer's thanks are due to his assistant, Mr F. J. Aylett, for the skilful manner in which he took charge of the animals and for the care with which he carried out the analytical work in connection with the digestibility trials.

Table I. *Details of rations.*

Period	Daily ration gm.	Dry matter in ration gm.
Green oats and tares	4000	1299.0
Oat and tare hay	1000	839.6
Oat and tare silage	3000	819.0

In planning the rations for the trials, it was, for obvious reasons, intended that the sheep should receive roughly the same weight of dry matter per day throughout. This object, however, was not attained for the following reasons. The first moisture determinations carried out on preliminary samples of the green oats and tares showed them to have a dry matter content of about 27 per cent. A diet of 4000 gm. of this fodder contained therefore about 1080 gm. of dry matter. By the end of the period, however, the dry matter content of the crop rose to over 31 per cent, and the mean percentage for the analytical period was

¹ For fuller information on this point, see Crowther and Woodman, *Journ. of Agric. Sci.* **8**, 434, 1917.

32.47 per cent. The dryness of the crop was probably connected with the droughty season during which it was approaching maturity. The consequence of this gradual increase in the percentage of dry matter was that the sheep received a larger allowance of dry matter during the period than was intended. Further, it was noted during the preliminary feeding of the oat and tare hay, that one of the sheep was unable to consume comfortably more than about 1000 gm. per day. There was no alternative, therefore, but to cut down the daily ration to this amount, and as a consequence, the amount of silage given in the daily ration during the third period had to be kept at the hay dry matter level, since it was desired, above all, to obtain a clean-cut comparison between the hay and the silage. The possible effect of these circumstances on the strict comparativeness of the trials will be discussed later.

GREEN OATS AND TARES PERIOD.

Condition of crop during trial. The crop consisted of autumn-sown oats and tares grown upon clay land. Equal quantities of grey winter oats and tares were planted, but the tares predominated at the time of the trial. Owing to the exceptionally dry summer, the crop was short in the straw and stood well. The oats were just coming into milk and the tares were in full flower. The condition of the crop was therefore almost ideal for cutting for hay, but was somewhat premature, according to the customary practice, for silage. It follows that the conditions of the trial slightly favoured the green oats and tares and the hay rather than the silage.

Table II. *Composition of green oats and tares composite sample (calculated to dry matter).*

		%
Crude protein	...	10.83
Ether extract	...	3.02
Nitrogen-free extractives	...	50.22
Crude fibre	...	28.12
Ash	...	7.81

Average moisture content of green oats and tares = 67.53 %.

Average amount of dry matter in green oats and tares per day = 1299.0 gm.

Table III. *Average weight and composition of faeces.*

	Sheep I gm.	Sheep II gm.
Weight of fresh faeces daily	1483	1576
Weight of dry matter daily	469.37	474.85

Table III (*cont.*). *Composition of dry matter.*

					SHEEP I %	SHEEP II %
Crude protein*	14.52	9.54
Ether extract	4.01	3.99
Nitrogen-free extractives	31.93	33.15
Crude fibre	40.18	40.84
Ash	12.36	12.48
Pepsin-HCl insoluble protein	5.24	5.17
* Crude protein as determined on <i>fresh</i> faeces					3.65	3.15

Table IV. *Digestibility of green oats and tares.*

Daily ration: 4000 gm. green oats and tares.

SHEEP I.								
		Total dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extrac- tives gm.	Crude fibre gm.	Ash gm.
Consumed (green oats and tares)	...	1299.00	1197.55	140.69	39.24	652.35	365.27	101.45
Voided	...	469.37	111.36	54.13*	18.82	149.87	188.59	58.01
Digested	...	829.63	786.19	86.56	20.42	502.48	176.68	43.44
Digestion coefficients, %		63.86	65.65	61.53	52.01	77.03	48.37	42.82
SHEEP II.								
Consumed (green oats and tares)	...	1299.00	1197.55	140.69	39.24	652.35	365.27	101.45
Voided	...	474.85	115.59	49.65*	18.95	157.41	193.93	59.26
Digested	...	824.15	781.96	91.04	20.29	494.94	171.34	42.19
Digestion coefficients, %		63.44	65.29	64.70	51.74	75.87	46.91	41.59
Mean digestion coeff., %		63.7	65.5	63.1	51.9	76.5	47.6	42.2

* Calculated on nitrogen of fresh faeces.

Protein digestibility corrected for metabolic nitrogen.

	SHEEP I	SHEEP II
Protein consumed, gm.	140.69	140.69
Pepsin-insoluble protein voided, gm.	24.59	24.55
Protein digested, gm.	116.10	116.14
Corrected digestion coefficient, %	82.52	82.55
Mean corrected digestion coefficient, %	82.5	

The satisfactory agreement displayed by the results for the two sheep will be noted and also that the agreement in the case of the protein digestion coefficients becomes still more marked after the large correction for metabolic nitrogen has been made.

Table V. *Percentages of digestible nutrients in green oats and tares (calculated to dry matter).*

By combining the results of the digestibility trial with the figures giving the composition of the green oats and tares, it is possible to calculate the percentages of digestible nutrients in the forage (dry matter).

	%
Crude protein*	6.83
Ether extract	1.57
Nitrogen-free extractives	38.42
Crude fibre	13.39
Production starch equivalent (Kellner) per 100 lbs. dry oats and tares ...	44.92

* Apparent digestion coefficient of protein employed in calculation.

Table VI. *Nitrogen balance during period and weights of sheep.*

Daily ration 4000 gm. green oats and tares	N consumed Av. per day gm.	N voided			Av. daily N balance gm.
		In faeces gm.	In urine gm.	Total gm.	
Sheep I	22.51	8.66	11.00	19.66	+2.85
Sheep II	22.51	7.94	12.18	20.12	+2.39
		SHEEP I		SHEEP II	
		st.	lb.	st.	lb.
		June 16, 1921	9 4	10 1	
		July 7, 1921	9 2 $\frac{1}{4}$	10 1	
		Change in weight	-1 $\frac{3}{4}$	0	

Whilst it is unsafe to base conclusions in connection with the utilisation of food protein from short period experiments, it is satisfactory to note that the forage diet provided a satisfactory maintenance ration for the animals.

OAT AND TARE HAY PERIOD.

Condition of fodder at time of feeding. Reference has already been made to the unfortunate circumstance which rendered necessary the rejection of the hay from the experimental plot and the employment instead of an unspoilt sample from another part of the field. The conditions under which the material was stacked have also been described. The small stack weighed about 5 cwt.; the outer portions, which were slightly spoilt by mould, were rejected and a slice of about 1 $\frac{1}{2}$ cwt. was cut down the middle (October 7, 1921). The sample was chaffed and the sampling and feeding were carried out in the manner already described.

The hay was mainly of a nice green colour, slightly bleached in places by the sun. It had no smell of heating in the stack, but retained the natural aroma of the herbage, characteristic of green hay. The crop was cut at an ideal time for hay, the oats being just in milk and the tares in

tall flower. The leaf had been well saved and the fodder could be described as "a useful sample of hay, although perhaps not of the very best quality¹." A mechanical analysis carried out on a representative sample of the hay showed it to contain roughly 30 per cent. by weight of oats and 70 per cent. of tares.

Table VII. *Composition of oat and tare hay composite sample (calculated to dry matter).*

	%
Crude protein	13.90
Ether extract	2.49
Nitrogen free extractives	15.84
Crude fibre	29.07
Ash	9.13
Average moisture content of oat and tare hay	16.04 %
Average amount of dry matter in oat and tare hay per day	839.6 gm

Attention has already been drawn to the reasons why the amount of dry matter fed per day during this period was smaller than in the preceding period. The high percentage of protein in the hay, as compared with the green forage, must also be noted. It would be expected that the hay dry matter would contain rather more protein than the dry matter of the green oats and tares, since, as a result of field fermentation and heating in the stack, losses of non-nitrogenous organic matter occur.

Table VIII. *Average weight and composition of faeces.*

	SHEEP I	SHEEP II
Weight of fresh faeces daily, gm.	604	701
Weight of dry matter daily, gm.	291.73	296.80

Composition of dry matter.

	SHEEP I	SHEEP II
	%	%
Crude protein*	12.43	12.54
Ether extract	3.77	3.77
Nitrogen-free extractives	37.34	37.83
Crude fibre	34.20	34.25
Ash	12.59	11.61
Pepsin-HCl insoluble protein	6.21	6.40
* Crude protein as determined on fresh faeces	6.02	5.43

The increase in the percentage of protein cannot, however, be wholly accounted for in this way, and the explanation is probably to be found in the fact that the hay was not, for reasons already stated, taken from

¹ The writer's colleague, Mr Arthur Amos, M.A., very kindly expressed his opinions regarding the quality of the hay and the silage used in these trials.

the experimental plot, but came from another part of the field where the growth of the crop was not quite uniform with that of the experimental crop. That the crop was not quite uniform within the limits of the experimental plot itself is evidenced by the fact that the silage was also slightly richer in protein than would be anticipated from a study of the losses of carbohydrates in the silo. It is not considered, however, that these circumstances materially affect the main conclusions to be drawn from the experiment, though they illustrate the extra difficulties introduced into work dealing with a *mixed* crop.

Table IX. *Digestibility of oat and tare hay.*

Daily ration: 1000 gm. oat and tare hay.

SHEEP I.

	Total dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extra- ctives gm.	Crude fibre gm.	Ash gm.
Consumed (oat and tare hay)	839.60	762.94	116.70	17.55	384.62	244.07	76.66
Voided	291.73	255.00	36.18*	11.00	108.84	99.77	36.73
Digested	547.87	507.94	80.52	6.55	275.78	144.30	39.93
Digestion coefficients, %	65.25	66.58	69.00	37.32	71.70	59.12	52.09

SHEEP II.

Consumed (oat and tare hay)	839.60	762.94	116.70	17.55	384.62	244.07	76.66
Voided	296.80	262.34	38.06*	11.19	112.28	101.65	34.46
Digested	542.80	500.60	78.64	6.36	272.34	142.42	42.20
Digestion coefficients, %	64.65	65.62	67.39	36.24	70.81	58.35	55.05
Mean digestion coeff., %	65.0	66.1	68.2	36.8	71.3	58.7	53.6

* Calculated on nitrogen in *fresh* faeces.*Correction of protein digestibility for metabolic nitrogen.*

	SHEEP I	SHEEP II
Protein consumed, gm.	116.70	116.70
Pepsin-insoluble protein voided, gm. ...	18.12	18.99
Protein digested, gm.	98.58	97.71
Corrected digestion coefficient, %	84.48	83.73
Mean corrected digestion coefficient, %	84.1	

The results again show good agreement between the two sheep. They will be discussed in detail at a later stage.

Table X. *Percentages of digestible nutrients in oat and tare hay sample (calculated to dry matter).*

		o
Crude protein*	...	9.48
Ether extract	...	0.77
Nitrogen-free extractives	...	32.66
Crude fibre	...	17.06

Production starch equivalent (Kellner) per 100 lbs. dry oat and tare hay = 43.24.

* Apparent digestion coefficient used in calculation.

Table XI. *Nitrogen balance during period and weights of sheep.*

Daily ration 1000 gm. oat and tare hay	N consumed Av. per day gm.	N voided			Av. daily N balance gm.
		In faeces gm.	In urine gm.	Total gm.	
Sheep I	18.67	5.79	11.23	17.02	+ 1.65
Sheep II	18.67	6.09	12.18	18.27	+ 0.40

	SHEEP I		SHEEP II	
	st.	lb.	st.	lb.
Oct. 13, 1921	9	4	10	8
Nov. 3, 1921	9	1	9	12
Change in weight	-3		-10	

It is scarcely a matter for surprise that the sheep during this period lost a little weight, since the ration was scarcely up to maintenance requirements, owing to the necessity which arose of having to cut down the amount of hay to secure complete consumption. Both sheep, however, showed a slight retention of protein during the trial.

OAT AND TARE SILAGE PERIOD.

Quality of silage used in experiment. The condition of the crop at the time of cutting (June 23, 1921) has already been described. The material was carted within three hours of cutting, so that little or no wilting was allowed to take place. The silage was made in a miniature wooden silo¹, which was 4 feet in diameter and 6 feet high and rested on a foundation of gault clay. The forage was first cut by the usual chaff-cutter and then filled into the small silo, precautions being taken that the material settled down compactly. A thick layer of soil was placed on top. The silo was opened on Nov. 8, 1921, and after rejecting the small amount of waste

¹ A number of such experimental silos have been erected on the Howe Hill Farm in connection with work being carried out by Mr A. Amos and the writer on the making of silage under controlled conditions, an account of which will be published shortly.

material on top, silage of excellent quality was encountered. It possessed a good green colour and a pleasant "fruity" odour, the smell of butyric acid being entirely absent. The silage as fed contained very few tare pods and no tare seeds, nor did the oat husks contain any solid food material. The sheep consumed it readily; the surplus was fed to stock, and it was observed that they ate it with relish and thrived upon it.

Fresh samples of silage for feeding were taken every day and the top of the silo was covered by a tarpaulin during the experiment. As the trial proceeded, the quality of the silage fell off slightly, owing probably to the slowness with which it was being used up. The last portions were not quite so green and "fruity," but still were of good quality and quite free from butyric acid.

Analysis of silage extract. In order to gain some insight into the nature of the changes which had occurred during ensilage of the green crop, aqueous extracts of the silage were submitted to analysis. A 200 gm. sample of the silage was submitted to extraction by shaking for four hours in a shaking machine with 600 c.c. of distilled water. The extract was filtered first through linen, the residue being well squeezed out, and then through a filter paper. 150 c.c. of the aqueous extract were made up to 500 c.c. with alcohol. This occasioned the separation of a small amount of precipitate, which settled readily, and the resultant clear alcohol liquid was submitted to analysis by the Foreman titration method¹. Fuller details regarding the analysis of silage extracts will be given in another communication.

Table XII. *Analysis of silage extract.*

(The data refer to 100 gm. of the *fresh* silage—moisture content = 72.9 %.)

	c.c. N/10
Total acid radicles (free and combined) ...	316.1
Amino acids and amides of asparagine type	104.2
Total organic acids of lactic and acetic type	211.9
Organic acids volatile in steam ...	56.6
Non-volatile organic acids ...	155.3
Volatile bases ...	18.1

Calculated as acetic acid, the percentage of volatile organic acids in the fresh silage works out at 0.34 per cent. This, of course, is reckoned as moisture by the customary method of determining dry matter, but allowance has been made for it in all the data tabulated here in connection with silage digestibility. To do this involved slight assumptions, which, however, could only possibly affect the result to an inappreciable extent.

¹ Foreman, *Bioch. Journ.* **14**, 451, 1920.

*Oat and Tare Silage*Table XIII. *Composition of oat and tare silage composite sample (calculated to moisture-free material).*

	%
Crude protein	12.55
Ether extract	4.32
Nitrogen-free extractives	45.57
Crude fibre	29.44
Ash	8.12

Average moisture content of oat and tare silage = 72.70 %.

Average amount of dry matter in oat and tare silage per day = 819.0 gm.

Table XIV. *Average weight and composition of faeces.*

	SHEEP I	SHEEP II
Weight of fresh faeces daily, gm.	569	631
Weight of dry matter daily, gm.	291.73	297.10
Composition of dry matter	%	%
Crude protein*	12.02	11.00
Ether extract	3.27	3.12
Nitrogen-free extractives	37.15	37.75
Crude fibre	35.03	35.23
Ash	12.53	12.90
Pepsin-insoluble protein	5.76	5.65
*Crude protein as determined on fresh faeces	6.38	5.61

Table XV. *Digestibility of oat and tare silage.*

Daily ration: 3000 gm. silage.

SHEEP I.

	Total dry matter gm.	Organic matter gm.	Crude protein gm.	Ether extract gm.	N-free extractives gm.	Crude fibre gm.	Ash gm.
Consumed (oat and tare silage)	819.00	752.50	102.78	35.38	373.23	241.11	66.50
Voided	291.73	255.18	36.25*	9.54	108.38	102.19	36.55
Digested	527.27	497.32	66.53	25.84	264.85	138.92	29.95
Digestion coefficients, %	64.38	66.09	64.73	73.01	70.96	57.62	45.04

SHEEP II.

Consumed (oat and tare silage)	819.00	752.50	102.78	35.38	373.23	241.11	66.50
Voided	297.10	258.77	35.56*	9.27	112.16	101.66	38.33
Digested	521.90	493.73	67.22	26.11	261.07	139.45	28.17
Digestion coefficients, %	63.72	65.61	65.40	73.80	69.95	56.59	42.36
Mean digestion coeff., %	64.1	65.9	65.1	73.4	70.5	57.1	43.7

* Calculated on nitrogen of fresh faeces.

The percentage of ether extract is not as large as would be expected from a study of the figures obtained for the organic acids in the silage extract. The difference is not wholly accounted for by the fact that a portion of the acids must exist in combination with bases, and it seems to indicate the occurrence in the silage of substances of an acidic nature which are not extracted by ether. Evidence that this might be the case was obtained during the titration of the extract with $N/10$ alkali. As the neutrality point was approached, a yellow colour developed in the originally almost water-clear solution. This point is being investigated further.

Protein digestibility corrected for metabolic nitrogen.

	SHEEP I	SHEEP II
Protein consumed, gm.	102.78	102.78
Pepsin-insoluble protein voided, gm. ...	16.80	16.79
Protein digested, gm.	85.98	85.99
Corrected digestion coefficient, %	83.66	83.66
Mean corrected digestion coefficient %	83.7	

As in the other two trials, there is little fault to find with the agreement shown by the two sets of coefficients.

Table XVI. *Percentages of digestible nutrients in the oat and tare silage sample (calculated to dry matter).*

	%
Crude protein	8.17
Ether extract	3.17
Nitrogen-free extractives	32.13
Crude fibre	16.81
Ash	3.55

Production starch equivalent (Kellner) per 100 lbs. dry silage = 45.59.

Table XVII. *Nitrogen balance during period and weights of sheep.*

Daily ration 3000 gm. oat and tare silage	N consumed Av. per day	N voided			Av. daily N balance
		In faeces	In urine	Total	
	gm.	gm.	gm.	gm.	gm.
Sheep I	16.45	5.80	9.88	15.68	+ 0.77
Sheep II	16.45	5.69	10.40	16.09	+ 0.36

	SHEEP I	SHEEP II
	st. lb.	st. lb.
Nov. 14, 1921	9 6	10 5
Dec. 5, 1921	8 12	9 8
Change in weight	- 8	- 11

Both sheep lost weight in this period, but no conclusions can be drawn from this as to the relative feeding values of hay and silage. The

periods were too short and the sheep were not on controlled diets previous to the different trials. The sheep would readily have consumed a heavier diet of silage, but it was desired to keep the amount of dry matter consumed per day roughly the same as in the hay period. Both animals were roughly in nitrogenous equilibrium during this period.

Table XVIII. *Summary of digestibility results.*

1. Comparison of digestion coefficients.

	Green oats and tares	Oat and tare hay	Oat and tare silage	Oat and tare silage (1920-21)
	%	%	%	%
Dry matter	63.7	65.0	64.4	55.3
Organic matter	65.5	66.1	65.9	55.8
Crude protein (apparent)	63.4	68.2	65.4	67.2
Crude protein (corrected)	82.5	84.1	83.7	
Ether extract	51.9	36.8	73.4	78.9
Nitrogen-free extractives	76.5	74.3	70.5	52.2
Crude fibre	47.6	58.7	57.4	49.7
Ash	42.2	53.6	43.7	50.2

2. Percentages of digestible nutrients (calculated to dry matter) and starch equivalents.

	Green oats and tares	Oat and tare hay	Oat and tare silage	Oat and tare silage (1920-21)
	%	%	%	%
Crude protein	6.83	9.48	8.17	10.91
Ether extract	1.57	0.77	3.17	3.35
Nitrogen-free extractives	38.42	32.66	32.13	19.47
Crude fibre	13.39	17.06	16.81	16.39
Production starch equivalent per 100 lbs. of dry fodder	11.92	43.24	45.59	33.4

Discussion of results. In the fourth column are given the figures obtained from digestibility trials with the same sheep on the previous year's crop of oat and tare silage. These results were not corrected for the volatile acid content of the silage. They differ materially from the results obtained in the present investigation, the previous year's silage possessing on the whole a markedly lower digestibility. This difference comes out strikingly in the cases of the dry matter, organic matter, nitrogen-free extractives and fibre, whilst the protein and ether extract fractions possess similar digestibilities. The previous year's silage was much richer in protein (16.23 per cent. on dry matter) than the 1921-22 crop (12.55 per cent.), and this is reflected in the table giving the amounts of digestible nutrients. The wide difference in the amounts of digestible carbohydrates in the two silage samples is also noteworthy, whilst the lower nutritive value of the previous year's silage is evidenced by the difference between the values of the starch equivalents.

It is of interest to examine the possible reasons for these wide variations in the results for the two silages, since obviously a practical point of some importance is involved. The differences in the procedures by which the two crops were produced are noted below in parallel columns.

SILAGE 1920-21.

- (1) Black winter oats sown.
- (2) Crop cut on July 12, 1920. It was quite mature. Oats had just passed milk stage and tares were well seeded.
- (3) Crop allowed to wilt one or two days before carting.
- (4) Silage made in commercial silo; maximum temperature of fermentation was 35° C. Silage brown in colour with somewhat pungent odour.
- (5) Seeds of both oats and tares in silage contained much solid food material.

SILAGE 1921-22.

- (1) Grey winter oats sown.
- (2) Crop cut on June 23, 1921. Crop was immature and ideal for hay. Oats were just coming into milk and tares were in full flower.
- (3) Crop carted within three hours of cutting.
- (4) Silage made in miniature silo. Temperature of fermentation did not exceed 25° C. Silage green in colour with pleasant fruity smell.
- (5) Silage contained very few tare pods and no tare seeds, and oat husks did not contain solid food material.

The first set of conditions is customarily regarded as ideal for silage. The results of this investigation, however, indicate that early cutting and carting without wilting may lead to a great gain in palatability, digestibility and nutritive value of the silage, although, of course, the actual weight of forage carted may be somewhat smaller per acre.

The chief results obtained in the present experiment on the comparative digestibilities of the three types of fodder are, with the exception of the protein figures, in fair agreement with the results obtained in American investigations with corn forage. These results, which are the averages from a large number of trials, not necessarily strictly comparative, have been summarised earlier in the paper. It will be noted that the digestibilities of the total dry matter, total organic matter and crude protein are of a similar order in all three cases. The uncorrected figures for protein digestibility indicated that the hay protein was distinctly more digestible than the protein of the silage and the green fodder, but on taking into account the metabolic nitrogen of the faeces, the inequalities are almost wiped out.

It would be anticipated that striking differences would occur in the digestion coefficients of the ether extracts. The ether extract of the green forage is about half absorbed; in the case of the hay, the availability sinks to about 37 per cent., whilst with the silage ether extract, which contains the easily assimilated organic acids, the relatively high figure of 73 per cent. is reached. It is not to be assumed, however, that the organic acids of the silage possess the nutritive qualities of the soluble carbohydrates of the green fodder from which they have arisen during ensilage.

Furthermore, it must be borne in mind that in not one of the three cases does the ether extract consist wholly of true fat, and also that the fat digestion coefficients are liable to be subject to error, in view of the fact that the faeces always contain ether soluble material arising from metabolic products and not from actual food residues.

The nitrogen-free extractives of the hay and the silage are approximately of equal digestibility, although in both cases the digestibility is lower than that of the corresponding fraction of the green forage. The depression of digestibility in this respect during the conversion of the crop into hay and silage is not, however, so great as has sometimes been supposed.

It is interesting to note that the fibre constituent of the hay and silage is almost equally digested, whereas that of the green crop possesses an appreciably lower digestibility. This finding confirms the supposition that heating in the stack and the silo leads to a definite increase in the digestibility of the crude fibre. In view of the fact that such fodders contain relatively large amounts of fibre, this increase of digestibility becomes of considerable significance.

Attention should be called to the fact that whereas in the hay and silage periods almost equal amounts of dry matter were fed per day, yet in the green oats and tares period a much larger allowance of dry matter, for reasons already gone into, was consumed by the sheep. It is well known that animals tend to digest their food with somewhat less completeness when the ration undergoes any marked increase in bulk. This variation is not necessarily very pronounced. Indeed, if it were, then the digestion coefficients based on feeding definite rations (usually sub-maintenance in such tests) could only possess a limited value. It is only fair, however, in comparing the green fodder digestion coefficients with those of the hay and the silage, to regard them as being minimum values, and to assume that if a ration of green fodder more comparable in dry matter content with the hay and silage rations had been fed, slightly higher values would have been obtained. This does not affect greatly the main conclusion that the digestibility of the hay and silage dry matter compares very favourably with that of the green fodder dry matter.

The findings outlined above are substantially confirmed by a study of the tabulated starch equivalents, which give the nutritive value of 100 lbs. dry fodder for production in terms of lbs. of starch. If anything, these results point slightly in favour of the silage.

The results giving the actual percentages of digestible nutrients in the dry foodstuffs, whilst not strictly comparable owing to slight non-

uniformity in the growth of the oat and tare crop, show clearly that both hay and silage contain somewhat more digestible protein and fibre and rather less digestible nitrogen-free extractives than the green crop. The amounts of digestible ether extract vary within wide limits, the hay figure being very low and the silage figure relatively high.

In commenting on the decrease of digestibility which is assumed to occur when a green crop is ensiled, Henry and Morrison¹ write: "The exceedingly favourable results from silage feeding are therefore due to the palatability of the silage, its beneficial effect on the health of the animals and the fact that less feed is wasted than when dry fodder is used." The results of this investigation indicate, however, that a contributory factor of great importance is the fact that the silage possesses a digestibility and a nutritive value which are only slightly, if at all, inferior to those possessed by the green forage from which it has been produced.

COMPARISON OF METABOLISABLE ENERGY OF GREEN OATS AND TARES, OAT AND TARE HAY AND OAT AND TARE SILAGE.

In order to extend the comparisons already outlined, it was decided to make determinations of the metabolisable energy of the three types of fodder. The metabolisable energy may be regarded as that portion of the gross energy of a foodstuff which is available for utilisation in the body of the animal; it does not, however, represent the true value of the foodstuff for general production purposes, since further deductions are necessary in allowing for the energy used up in the processes of mastication and digestion. The metabolisable energy is ascertained by deducting from the gross energy of the foodstuff the losses of energy from the body in the form of the liquid, solid and gaseous excreta; its determination involves, therefore, the carrying out of bomb calorimetric experiments on the foodstuff, dry faeces and dry matter of the urine. The course of the determinations will be gathered from a study of the following tables.

Table XIX. *Details of urine output during trials.*

Period	SHEEP I		SHEEP II	
	Average daily amount	Mean dry matter	Average daily amount	Mean dry matter
	c.c.	%	c.c.	%
Green oats and tares	1140	6.18	1288	5.68
Oat and tare hay	1826	3.60	954	7.64
Oat and tare silage	2051	2.63	1812	3.25

¹ *Feeds and Feeding*, p. 51, 1917.

*Oat and Tare Silage*Table XX. *Dry matter balances per 100 gm. dry matter consumed.*

Period	Dry matter consumed gm.	SHEEP I		SHEEP II	
		Average daily dry matter of faeces	Average daily dry matter of urine	Average daily dry matter of faeces	Average daily dry matter of urine
		gm.	gm.	gm.	gm.
Green oats and tares	100	36.13	5.42	36.55	5.63
Oat and tare hay	100	34.75	7.83	35.35	8.68
Oat and tare silage	100	35.62	6.59	36.28	7.19

Table XXI. *Heats of combustion per gm. of dry matter.*

Green oats and tares	4214 cal.
Oat and tare hay	4211 ..
Oat and tare silage*	4338.3 ..

Period	SHEEP I		SHEEP II	
	Faeces cals.	Urine dry matter cals.	Faeces cals.	Urine dry matter cals.
Green oats and tares	4317	1959	4326	2659
Oat and tare hay ...	4511	1708	4441	1890
Oat and tare silage	4517	2100	4413	2216

* Allowance made for volatile acids as acetic acid (heat of combustion of acetic acid = 3490 cal.,—Berthelot).

Table XXII. *Energy balances per 100 gm. dry matter consumed.*

Period	Gross energy of foodstuff Cals.	SHEEP I		SHEEP II	
		Loss of energy in faeces Cals.	Loss of energy in urine Cals.	Loss of energy in faeces Cals.	Loss of energy in urine Cals.
Green oats and tares	424.4	155.97	10.62	158.12	11.59
Oat and tare hay	421.4	156.76	13.37	156.99	16.40
Oat and tare silage	433.83	160.89	13.84	160.10	15.93

The above figures enable the metabolisable energy per 100 lbs. of dry foodstuff to be calculated. The results are expressed in therms (1 therm = 1000 Cals.).

Table XXIII. *Metabolisable energy per 100 lbs. dry foodstuff.*

	Green oats and tares therms	Oat and tare hay therms	Oat and tare silage therms
Sheep I	116.94	113.84	117.53
Sheep II	115.53	112.36	116.93
Mean	116.23	113.10	117.23

It will be noted that the agreement between the results for the sheep is again quite satisfactory. The above figures, however, still require correction for the energy lost in the gaseous excreta, which could not, of

course, be measured under the conditions of this experiment. Recourse was therefore had to the correction figure given by Armsby¹, namely, a deduction of 60·1 Cals., was made per 100 gm. of digested carbohydrates. In this way, the following figures were obtained, which are compared with figures given in the second column, which have been calculated by the use of Armsby's factor by multiplying the digestible organic matter by 1·588.

Table XXIV. *Corrected metabolisable energies compared with calculated values (per 100 lbs. dry fodder).*

	Experimental figure therms	Calculated figure therms
Green oats and tares	102·10	95·61
Oat and tare hay	99·55	95·23
Oat and tare silage	103·89	95·72

The values obtained by the use of Armsby's factor are thus uniformly lower than the experimental figures.

The above results are in harmony with the conclusions drawn from a study of the digestibility and nutritive value of the fodders. As between the hay and the silage, the advantage appears to rest with the silage. In order to obtain the actual productive energy of the foodstuffs (Net Energy Values), it would be necessary to make a further subtraction corresponding with the energy used up in mastication and digestion and ultimately lost as heat from the body (Increment of Heat Production). It may be presumed that this consideration would operate still further to the advantage of the silage, since it is reasonable to suppose that less energy will be used up in the mastication and digestion of the soft and succulent silage, than in the corresponding processes with the dry and coarse hay fodder.

In conclusion, the writer would like to take this opportunity of acknowledging his indebtedness to Professor T. B. Wood, C.B.E., M.A., F.R.S., for much valuable advice during the course of this investigation; also to Mr Arthur Amos, M.A., who not only supervised the making of the hay and the silage, but also supplied the writer with many interesting and useful details concerning the growing of the crop and the quality of the respective fodders.

¹ *Nutrition of Farm Animals*, p. 639.

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A NOTE ON THE CLASSIFICATION OF SOILS ON THE BASIS OF MECHANICAL ANALYSES.

By C. L. WHITTLES.

School of Agriculture, Cambridge.

(With Eleven Figures.)

ALTHOUGH many schemes have been proposed for a classification of soils¹, not any single one seems to have found general acceptance. It is, however, generally agreed that with any method, a final subdivision based on the texture of the soil is highly desirable, even though it only be the sands, loams, and clays of common parlance. Granted then that a classification based on mechanical analyses is called for—though not necessarily as the primary division into groups—it is here proposed to examine some of the various methods so far put forward for dealing with the results of mechanical analyses. Tables of analytical results in themselves are unwieldy and make decidedly uninteresting reading. The comparison of a large number of soils is a slow and tedious process, if it is accomplished only by a study of the figures.

Hope and Carpenter⁽²²⁾ have devised a method by which one can rapidly refer a soil to one of twelve types. In their classification the particles are classified into four groups, the limiting dimensions of which are given in Table I.

Table I. *Hope and Carpenter's Classification of Particles.*

Ingredient	Limiting diameter in mm.	
	Maximum	Minimum
1. Coarse sand	1.0	0.28
2. Fine sand	0.28	0.04
3. Silt	0.04	0.01
4. Fine silt and clay	0.01	—

Four broad divisions are distinguished according as one or other of these fractions occurs in the largest percentage. Each of these divisions is subdivided into three classes according as one or other of the three remaining fractions preponderates. Each ingredient is represented by a number (1, 2, 3 or 4), and the type is named by placing the division

¹ See Bibliography.

number first, followed by the appropriate class number. This is summarised in Table II. They point out that each type has a corresponding type into which it can merge by inappreciable degrees, the transformation being effected by interchange in the proportions in the ingredients which are of primary and secondary importance respectively, *e.g.* 1, 2 to 2, 1. On the other hand, they can diverge far from each other in character. Other relationships are pointed out of which the most important perhaps is the change in the secondary ingredient, as for example from 1, 2 to 1, 3.

Table II. *Hall and Carpenter's Classification of Soils.*

Primary ingredient (division)	Secondary ingredient (class)	Type
1. Coarse sand	(2. Fine sand	1, 2
	(3. Silt	1, 3
	(4. Fine silt and clay	1, 4
2. Fine sand	(1. Coarse sand	2, 1
	(3. Silt	2, 3
	(4. Fine silt and clay	2, 4
3. Silt	(1. Coarse sand	3, 1
	(2. Fine sand	3, 2
	(4. Fine silt and clay	3, 4
4. Fine silt and clay	(1. Coarse sand	4, 1
	(2. Fine sand	4, 2
	(3. Silt	4, 3

The earliest reports of mechanical analyses were often illustrated by photographs of the actual fractions obtained. The sand, silt, etc., were placed in small phials of uniform size, and the predominance of the bulk of the material in certain grades served to give a more vivid idea as to the meaning of the figures of the analysis. The method at that time had its value, for the purpose of a mechanical analysis was not so well known then as now. Hilgard's text-book (21) and the early bulletins of the U.S. Department of Agriculture contain illustrations of this type.

It is only a short step from the use of the actual fractions to that of shaded blocks. In Whitney's report on the tobacco soils of Maryland (57) a general summary of the types is given in diagrammatic form in which three grades are distinguished by different shading, viz. sand, silt and clay.

In order to compare several soils or soil types on the same diagram the method of plotting the percentage of each grade against an arbitrary scale of grades has frequently been adopted. Differences are here far more obvious than resemblances, and their correct interpretation is almost as difficult as that of the figures themselves. The plotting of the

summation percentage against either an arbitrary scale, a natural scale of mean diameters (5), or more conveniently the logarithms of the mean diameters, is to be preferred. By this means a very complete representation of the analysis can be made, and the gradual gradation from type to type clearly seen, but the method becomes unintelligible if any number of soils are plotted on one diagram. A number of soil types are shown in Fig. 1, where the summation percentages are plotted against the logarithms

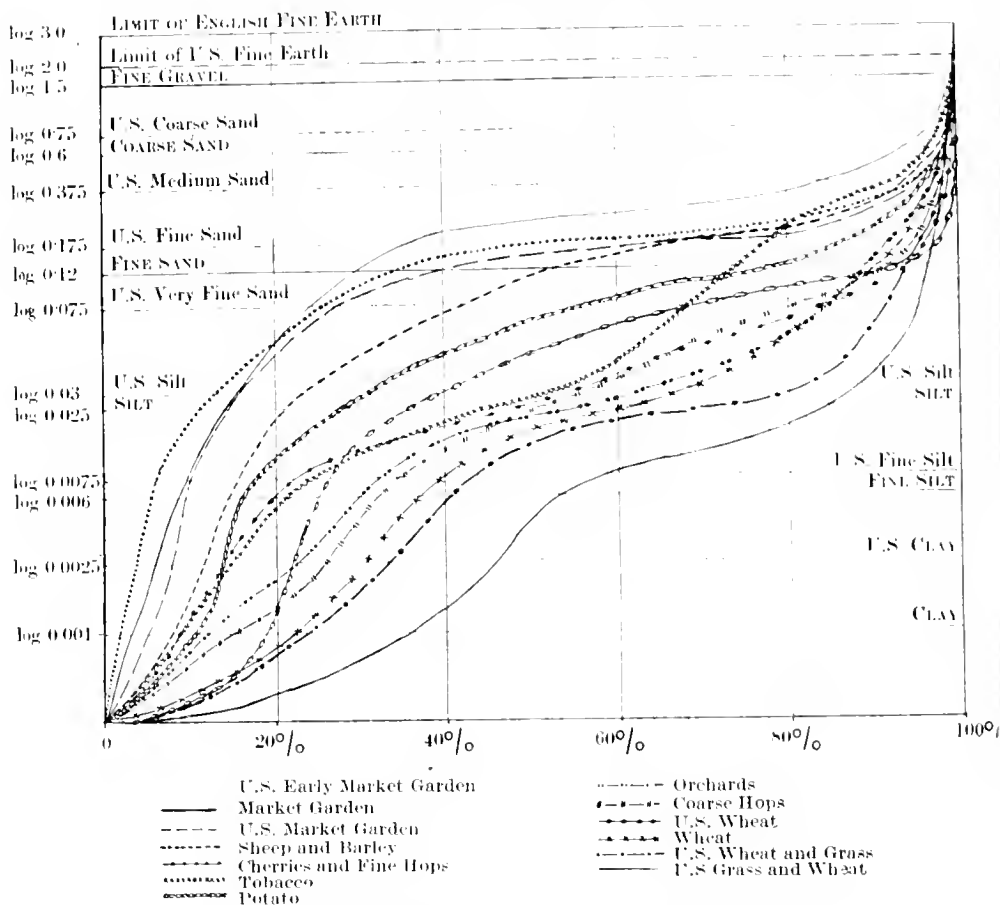


Fig. 1. Comparison of types by summation curves.

of the mean diameters of the groups, the points so obtained being joined up by a smooth curve. The value .0001 mm. has been taken as the lower limiting value of the dimensions of the clay group. This is purely an arbitrary proceeding. The proportion of "colloid" clay could here be

shown with great advantage. In this connection the use of ultra-filtration methods might give valuable information.

The curves for the lighter soils rise steeply and then flatten out, those for the heavier soils are flat at first and rise steeply later. Two particular applications of these curves are of importance:

(1) Two soils, analysed according to different systems of grouping of particles may be compared exactly.

(2) The analytical results may be transformed from one system to another by reading off the values at the points at which the curve cuts the mean diameter of the selected classes.

Baker(7) has devised two values for describing a soil from its mechanical analysis plotted in this way. This is of value for catalogue and descriptive purposes, but is not so well adapted for the preparation of drift maps as is the triangular method. In this method only three classes of particles can be considered, which may be conveniently termed Coarse, Medium and Fine respectively. The question now arises as to which two limiting values shall be chosen for the separation of the Medium class from the Coarse and Fine grades respectively. Considering Fig. 1 we find that:

U.S. Bureau of Soils take .03 and .0025.

Wilsdon(58) takes .025 and .001.

It is here suggested that, taking into consideration the known properties of the various grades, the curves are best characterised by their intercepts on the lines for .12 and .006. On this basis we have, on the usual English system:

Coarse (fine gravel + coarse sand)

Medium (fine sand + silt)

Fine (fine silt + clay).

TRIANGULAR METHODS.

In the method adopted by the U.S. Bureau of Soils the proportions of clay and silt are plotted along each of two axes at right angles. By joining the 100 points on these lines a right-angled triangle is formed. This is then conventionally divided up into compartments as shown in Fig. 2. The scheme of classification is given in Table III.

Wilsdon has suggested a modification of this method whereby the proportion of the third constituent—the sand—may be read off directly from the diagram. The percentages of sand, silt and clay are plotted on an equilateral triangle. The detailed procedure is given in connection

Table III. *U.S. Bureau of Soil's Classification.*

	Fine gravel 2-4 mm.	Coarse sand 1-5 mm.	Medium sand .5-2.5 mm.	Fine sand .25-10 mm.	Very fine sand .10-.05 mm.	Silt .05-.005 mm.	Clay -.005-0 mm.
	1	2	3	4	5	6	7
Coarse sand	More than 25 % (1+2) More than 50 % (1+2+3)					0-15 % Less than 20 % (6+7)	0-10 % (6+7)
Medium sand	Less than 20 % (1+2) More than 20 % (1+2+3)					0-15 % Less than 20 % (6+7)	0-10 % (6+7)
Fine sand	Less than 20 % (1+2+3)					0-15 % Less than 20 % (6+7)	0-10 % (6+7)
Sandy loam	More than 20 % (1+2+3)					10 %-35 % More than 20 % (6+7) Less than 50 % (6+7)	5 %-15 % (6+7)
Fine sandy loam	Less than 20 % (1+2+3)					10 %-35 % More than 20 % (6+7) Less than 50 % (6+7)	5 %-15 % (6+7)
Loam						Less than 15 %-25 % 55 % (6) More than 50 % (6+7)	
Silt loam						More than 15 %-25 % 55 % (6)	Less than 25 % (7)
Clay loam						25 %-55 % More than 60 % (6+7)	25 %-35 % (6+7)
Sandy clay						Less than 25 % (6) Less than 60 % (6+7)	More than 20 % (7)
Silt clay						More than 25 %-55 % (6)	35 % (7)
Clay							More than 35 % (7) More than 60 % (6+7)

with a further modification. Soils are classified into fourteen groups, the limiting proportions are given in Table IV, and illustrated graphically in Fig. 3.

Attention is directed to the following considerations in connection with this method:

1. Only a comparatively small area of the whole triangle is employed.
2. The crop types are not so well differentiated as in that of the U.S. Bureau of Soils.

Table IV. *Classification of Soils (Wilsdon).*

Description	Percentage		
	Sand (+0.04 mm.)	Silt (+0.002 mm.)	Clay (-0.002 mm.)
Sand	+75	-25	-25
Sandy loam I	+60	-40	-10
" " II	+60	-30	-20
Heavy sandy loam	-75	-20	-30
Loam I	+45	+30	-10
" II	-45	+45	-10
" III	-60	+20	-20
" IV	-50	+30	-20
" V	-40	+40	-20
Silt	-30	+50	-20
Heavy silt loam	-20	+50	-30
Clay loam I	-60	-40	-30
" II	+20	+30	-30
Clay	-70	-70	+30

A + sign is placed before a minimum limit, and a - sign before a maximum.

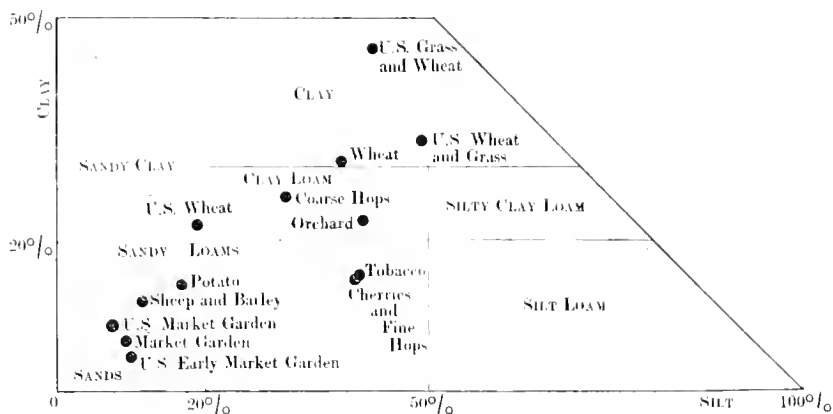


Fig. 2. Comparison of types by U.S. method.

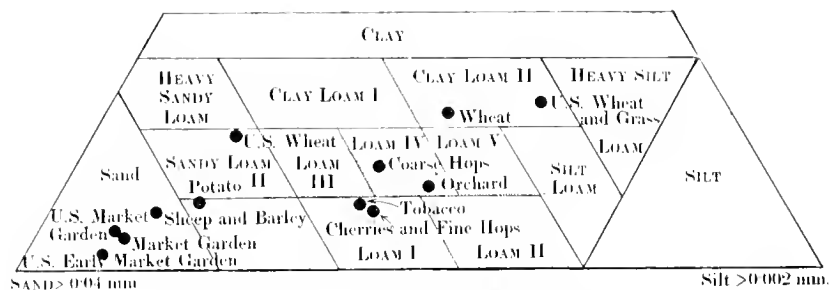


Fig. 3. Comparison of types by Wilsdon's method.

3. It is difficult to associate each of the three chosen groups with distinct properties in regard to their relation to the movements of water(49).

4. Luxmore(29) has shown that many properties of soils can be correlated with the proportion of particles having a maximum diameter of 0.01 mm.

5. Taking into account the insensible degrees by which any given type merges into another, are we justified in laying down limiting percentages for classes of soils? A soil with a given analysis behaving as a sandy loam in a district of deficient rainfall becomes a loam with a medium rainfall, and a heavy loam with a very high rainfall. The influence of the amounts of organic matter and of calcium carbonate cannot be ignored.

6. Different analysts, working on the same sample of soil frequently—as often as not—obtain discordant results for the clay and fine silt fractions, though they will agree as to the total amount of these two grades present.

7. The rapidity with which a large number of samples can be analysed if the determination of the clay is omitted is an important point.

8. The curves for the summation percentages are better characterised by the proposed limits.

The various limiting diameters of the three classes are shown in Table V.

Table V. *Classification of particles.*

System	Limits of diameters in millimetres					
	Coarse (sand)		Medium (silt)		Fine (clay)	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
American	2.0	0.05	0.05	0.005	0.005	—
Wilsdon	1.0	0.01	0.01	0.002	0.002	—
Proposed	3.0	0.2	0.2	0.01	0.01	—
[Or possibly	—	0.2	0.2	0.02	0.02	—]

Atterberg(44) has suggested the limits .2, .02 and .002, and there appears to be fairly strong evidence that a geometrical progression is the type of classification required. As English analysts do not recognise the limit 0.02, the nearest English point (0.01) has been adopted here provisionally. The result obtained with the types previously considered is shown in Fig. 1.

The actual procedure adopted for plotting the results can be seen from the following example:

Fine gravel	8.9	}	25.9. Coarse.
Coarse sand	17.0		
Fine sand	17.9	}	30.3. Medium.
Silt	12.4		
Fine silt	24.5	}	28.9. Fine
Clay	4.4		

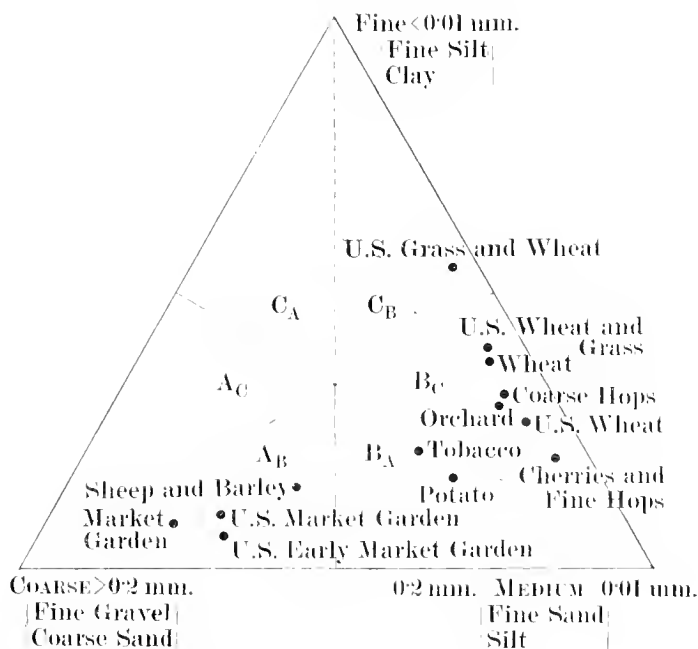


Fig. 4. Comparison of types by proposed method.

In Fig. 5 the side of the equilateral triangle ABC is 100 units, the apices represent 100 per cent. of the respective ingredients.

From B along BA cut off $BP = 25.9$ units

„ A „ AB „ $AQ = 30.3$ „

„ A „ AC „ $AR = 28.9$ „

From P , Q and R draw PX , QY and RZ parallel with BC , AC and AB respectively to form the triangle pqr .

Then any point on the line PX represents 25.9 per cent. of the coarse particles, on QY 30.3 per cent. of the medium particles, and on RZ 28.9 per cent. of the fine particles.

Then the centre S of the triangle pqr , obtained by bisecting the base angles is the required point. This construction avoids the necessity for raising the percentages arithmetically so that they total 100. In general they will be less than 100, for the losses on solution and ignition are not included in the amounts plotted.

Soils with a high content of organic matter or of calcium carbonate cannot be compared by their position on the triangle alone. By the employment of an arbitrary colour scale, the organic matter content, or the acidity expressed in terms of titration values or of hydrogen ion concentration could be shown simultaneously with its approximate mechanical analysis. Rainfall and other climatic data are obviously open to a similar method of treatment.

In order that the position of a point on the diagram may be rapidly interpreted, a diagram showing the proportions of each of the three ingredients present, by steps of 10 per cent. has been prepared. By super-imposing this on any of the soil diagrams the limits of the groups can rapidly be read off.

The amount of variation permissible in a mechanical analysis for survey purposes has been investigated by Robinson (39). The analyses of the two yields, (*a*) uniform, (*b*) too variable, are shown in Fig. 5. Attention is drawn to this in order that an idea may be obtained as to the value that is to be assigned to any amount of scatter in a diagram.

The general arrangement of the soil types is indicated in Fig. 4. The triangle has been divided up into three main divisions (marked in solid lines) according as one or other of the three ingredients predominates. Each division is subdivided into two classes (by dotted lines) according as one or other of the remaining two constituents is in excess. The inter-relationship between class and class is thus proportional to the length of the dividing line. They may merge into one another or diverge widely. For convenience of reference the classes may be named A_B , B_A , B_C , C_B , C_A and A_C , respectively as shown.

Fig. 6 shows a number of wheat soils from almost every geological formation. It will be noticed that they tend to be more or less concentrated around those selected by Hall and Russell as typical. The lighter soils of Norfolk on which wheat is grown, though the soils are not particularly well adapted to the crop, merge into the typical wheat group, which apparently lies near the boundary of B_C and C_B . The soils in C_B are on the whole more typically grass than arable (12, 16, 17, 19, 20, 29, 31, 36, 40).

Barley soils are illustrated in Fig. 7. The crop is grown on soils of the

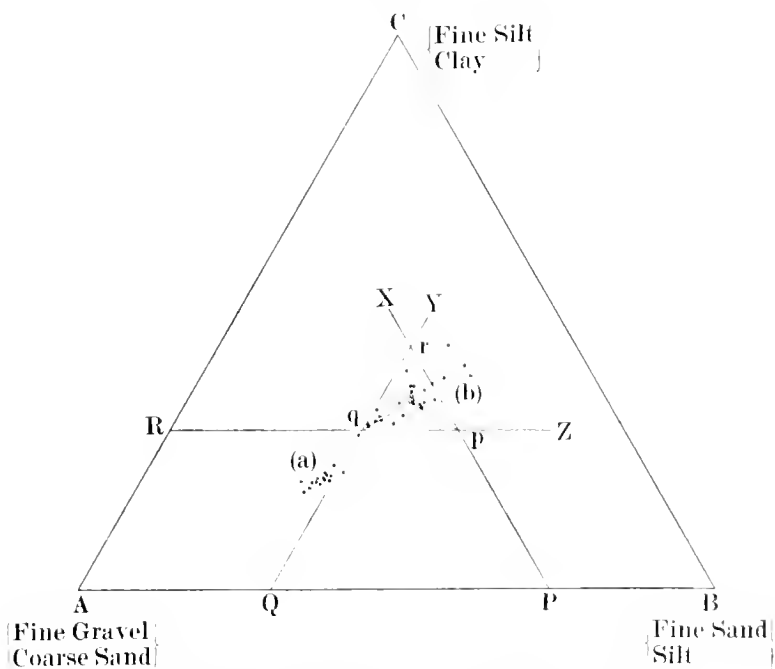


Fig. 5. Method of plotting, and degree of variation permissible.

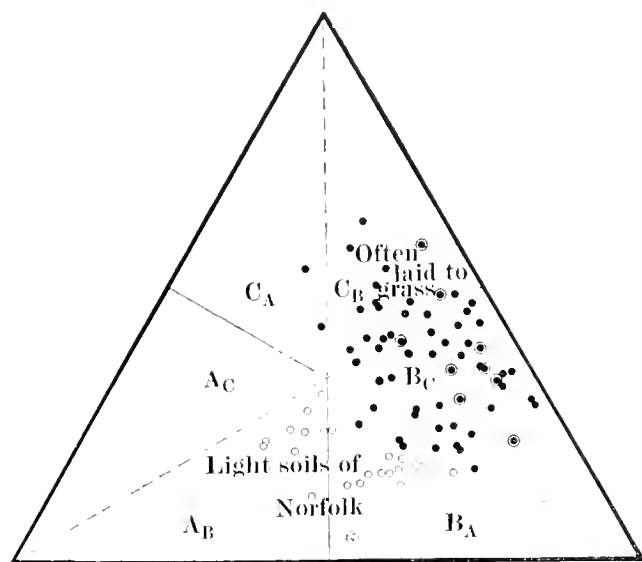


Fig. 6. Wheat soils.

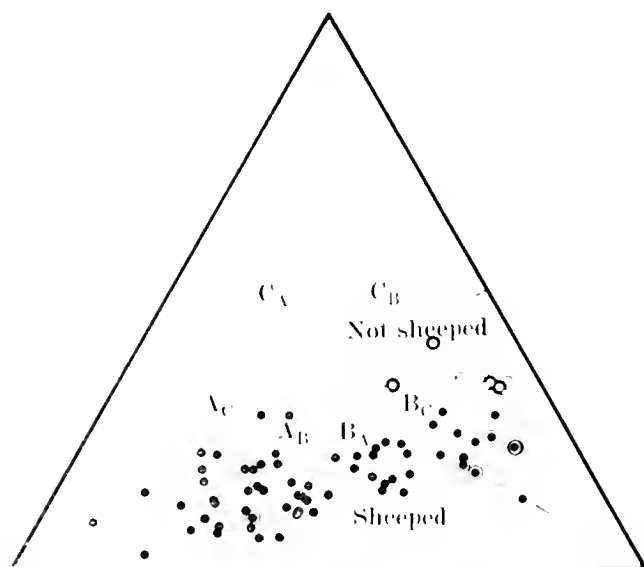


Fig. 7. Barley soils.

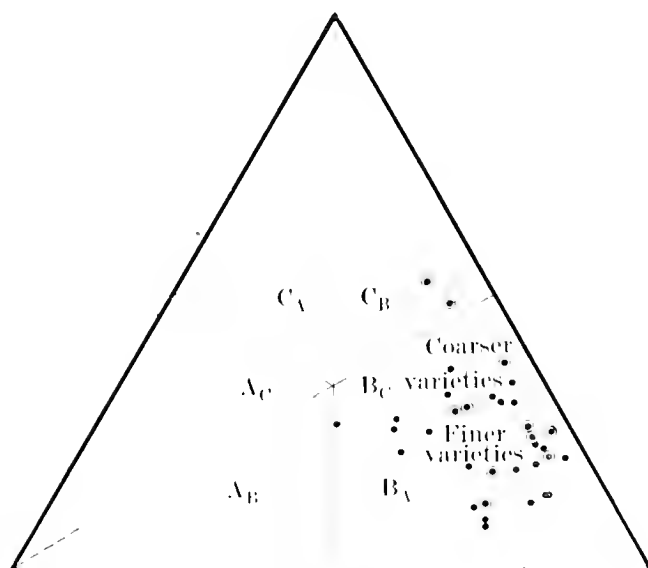


Fig. 8. Hop soils.

⊙ Typical Soils, quoted by Hall and Russell

• Other typical soils, from various sources

○ Soils on which crop is grown, though soil not really typical of the crop

⊙ Ditto (quoted by Hall and Russell)

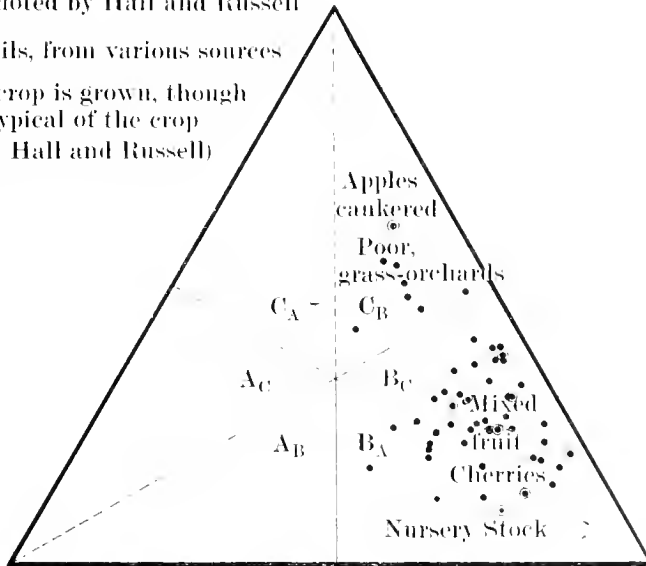


Fig. 9. Orchard soils.

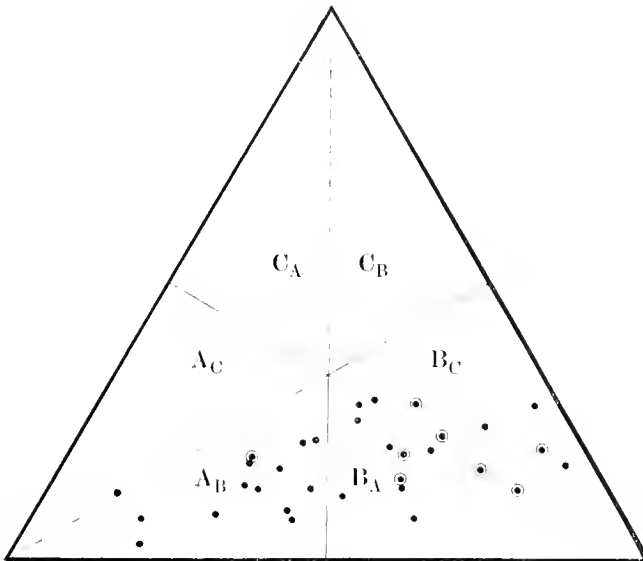


Fig. 10. Potato soils.

three classes A_B , B_A and B_C , but it is only the lighter soils of the latter class that can carry sheep.

Hop soils are illustrated in Fig. 8, and Orchard soils in Fig. 9. The comparison of these two types made by Hall and Russell (49) is clearly illustrated. The orchard soils in C_B , apart from the extreme case quoted by Hall and Russell from the Weald Clay, are all grassed orchards, and according to the Bristol Reports (13, 14, 55) suffer from canker to a greater or less extent. Evidently these soils are really too heavy for fruit.

In Fig. 10 a number of potato soils are shown. The limiting factor for a potato soil, low content of coarse silt, is not brought out very well by this method (1, 19, 20, 11).

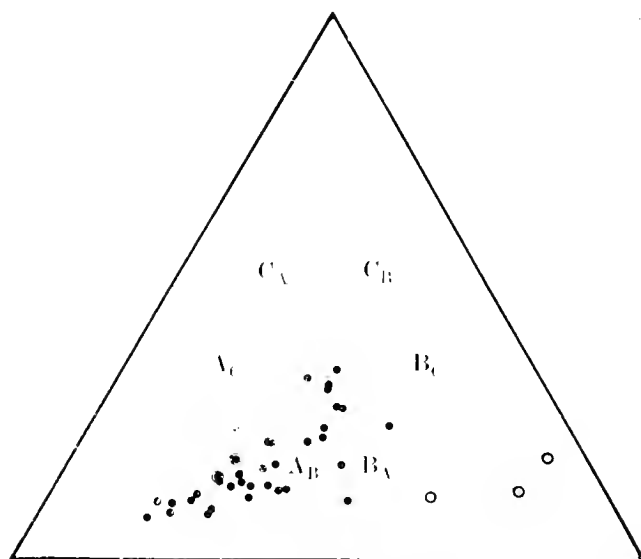


Fig. 11. Market garden soils.

The Biggleswade Market Garden soils (35) which are most typical are shown in Fig. 11. Those which have been utilised on account of economic reasons have been omitted. Hall and Russell's Merton Alluvial soils approximate more closely to the Biggleswade type than does the Weybridge, but these are both more of the Market Garden type than the Bagshot Windlesham and the Thanets from Swanley and Greenhythe.

APPLICATION TO THE PREPARATION OF MAPS.

If each of the three primary colours be taken to represent one of the three constituents (Coarse, Medium, and Fine), then to any position on the triangle is a corresponding definite colour, produced by a combination of the three primary colours in the same proportions. Any desired degree of differentiation may be attained by using a sufficiently large number of combinations. Steps of 5 per cent. would give 231 types, and this would be sufficiently accurate for all purposes, as will be seen from a consideration of Fig. 5. For the preparation of the drift maps of a district, steps of 10 per cent. giving 66 types would probably be sufficient. The proportion of stones and gravel, chalk, organic matter, etc., could be shown by dots or shading. Maps of the soil and subsoil prepared on these lines would be of great value.

In conclusion, the writer begs to tender his thanks to all those who have supplied him with data and other help.

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THE INFLUENCE OF SIZE AND CHARACTER OF SEED ON THE YIELD OF POTATOES.

BY REDCLIFFE N. SALAMAN, M.A., M.D.,

Barley, Herts.

(With Four Text-figures.)

THE results of a preliminary investigation on this subject¹, in which single plots were used, which were published in 1921, tended to show that the following inferences, in respect to the relation of size and type of tuber-set to crop, might be drawn.

1. The size of the crop varied directly with the weight of the set up to a certain point, viz. 2 ozs., the crop then sinking slightly to a more or less constant level, whilst the weight of the set increased.

2. The crop resulting from the planting of large sets with secondary outgrowths exceeded that from all other types of sets and was as much as 25 per cent. more than that derived from sets of equal size without outgrowths.

3. The proportion of heavy ware in a crop varied inversely with the size of the tuber-set, and was not materially affected by the existence or otherwise of outgrowths on the set.

4. The tendency to form outgrowths was not conveyed by tuber.

The problem was re-investigated in 1921. As in the previous year, the Potato used was the variety Barley Bounty², the crop of which had been grown in Scotland the previous season. The experimental plot was situated on the writer's farm at Barley, on a piece of ground which sloped gradually from east to west. The western end was slightly more clayey than the eastern portion, but on the whole the soil was of a uniform type of loam and had been specially chosen on that account. The area of the ground on which the experimental sets were planted was 17·5 feet x 400 = 777 sq. yards. It was deeply cultivated; no stable manure was

¹ Salaman, R. N., *Journ. of the Ministry of Agric.* Vol. XXVIII, April 1921.

² This is a wart immune variety raised by the author in 1911 which is very resistant to roll and mosaic and partially so to *Phytophthora*. It is not yet on the market.

used, but it was sown with the following artificials: superphosphate 84 lbs., sulphate of ammonia 20 lbs., and kainit 28 lbs. The guard rows were treated in like manner.

Eleven classes of seed tubers were selected, boxed and sprouted, on the same date. In each class the seed was weighed, pound by pound, so that there was a check both as to size and individual weight. Thus, eliminating as far as possible, any source of error arising from inequality of seed within each class, an error which has vitiated some otherwise valuable work in past years.

Class A.	Tubers weighing 0.6 oz. or 26 to 1 lb.
.. B. 1.33 ozs. .. 12 ..
.. C. 2.0 8 ..
.. D. 2.66 6 ..
.. E. 4.0 4 ..
.. F. 5.33 3 ..
.. G.	Mixed unselected seed tubers.
.. H.	Whole tubers with outgrowths weighing 2 ozs. each.
.. I.	The crown ends from tubers with lateral outgrowths weighing 1.66 ozs. each.
.. J.	Cut sets with outgrowths weighing 2 ozs. each.
.. K.	Cut sets with outgrowths, weighing 1.25 ozs. each.

All tuber-sets presented short, strong sprouts and no blind sets were planted.

Of these classes, B, C, D and G were present in sufficient quantity to plant five rows of 100 tubers each. A and E filled three rows respectively of 100 tubers each. F was sufficient for one full row and part of another. H, J and K were present only in sufficient quantities to plant part rows of each. It should be noted that in a standard plot, viz. a row 100 feet long, not only was the number of sets the same, but in respect to any class, the total weight of sets was exactly the same.

The experiment was planned on the checker-board system (see Fig. 1), but in place of square plots, single rows were used. As the variety was the same throughout, no need was felt for guard rows of some neutral variety between the rows, but surrounding the whole experiment were rows of the variety Golden Wonder, each section containing 100 tubers. On the northern side two rows of Golden Wonder were succeeded by a crop of barley, on the south the two rows were followed by more potatoes.

The rows were exactly two and a half feet apart and the tubers were sown by hand, the distance between the centres of every two consecutive tubers being one foot. The spacing between tubers was carefully controlled by actual measurement as each tuber was planted. The covering in, first hoeing, and earthing up were done by horse labour.

184 *Influence of Seed Weight, etc. on the Potato Crop*

The plot was so arranged that these seven rows, with their two border rows on either side, eleven in all, ran from east to west. Each of the seven rows were subdivided by stakes at intervals of 100 feet making four such groups of 100 feet on end.

The smaller lots were all planted in the lowest group of rows and were so planted that F, A and K together contained 100 tubers and made one complete row, whilst H, J and I did the same in a neighbouring row.

Good intentions notwithstanding, the plot proved to be anything but equable in character. As the drought proceeded it became increasingly clear that the lowest lying, *i.e.* eastern end, was favoured by more residual moisture in its rather more friable soil, and probably by a greater precipitation of dew, the influence of which on the colour and size of the plants was exceedingly clear. On the other hand, there was no apparent difference in the soil condition from north to south. It is true that the outermost row of Golden Wonder against the crop of Barley was even more impoverished than its neighbour or either of the guard rows on the southern side of the plot, but this was undoubtedly due to the too close proximity of a vigorous cereal crop. *En passant* it should be noted that two guard rows are insufficient as a protection to an experimental plot against a foreign crop, but quite sufficient as against another potato crop.

Besides the exhausting effect of the drought, there was a frost on June 19th which nipped the leaves of several plants in all parts of the plot, whilst towards harvesting time surface caterpillars in search of moisture wrought considerable havoc on the tubers. No single set failed to produce a plant of some size.

From April 19th, the day the tubers were sown, until they were harvested in October, no rain whatever (barring a slight shower in October) fell on the experimental ground, although heavy rain had fallen elsewhere in the parish during August. The potatoes were raised by hand on October 19th, exactly six months after planting, weighed at once and stored in separate bags. The analysis of the crop was made in January of this year.

In Fig. 1 the plots are shown diagrammatically. In the fourth row, lots H and I in reality formed parts of the same row as J, and lots K and F parts of the same row as A. They are only placed in the diagram alongside each other for the sake of clearness. In each plot the return of crop per set, *i.e.* the hundredth part of the total crop, is shown blocked in black, the actual figures are given in Schedule I at the end of the

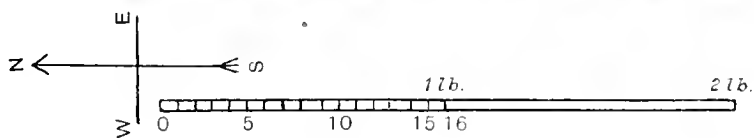
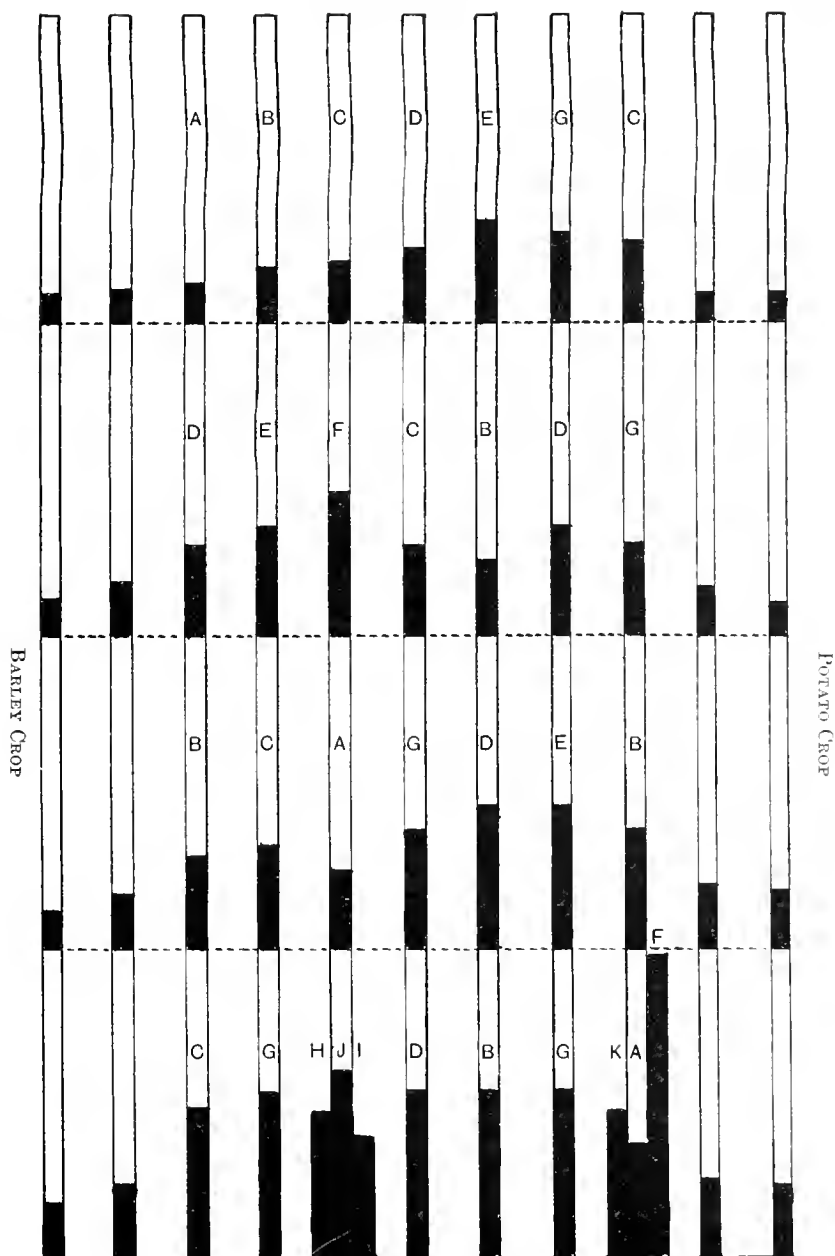


Fig. 1.

paper, in the case of the smaller lots in the lowest row, the return per tuber set is calculated after correcting for the lesser number of plants.

As there was no disease in the tubers at all, there being but very little in this part in 1921, and the variety itself exhibiting considerable resistance to *Phytophthora*, no allowance was needed for this possible source of error. If the diagram be studied it will appear that:

(a) There is much less variation in the crop return on the guard rows both as regards plots alongside and plots above or below each other, than there is in the case of the A-K series.

(b) That whilst yields in the outermost guard row on the north side are uniformly less in all four of its divisions than those of its inner neighbour, those of the latter do not materially differ from those of the two guard rows on the south side, which, in their turn, are almost completely alike.

(c) The variation between the upper and lower divisions in the guard rows, more especially the inner northern and the two southern rows, is such that the crop is gradually increased from east to west so that that of the latter is twice the value of that of the former rows. On the other hand, the returns of any of the four rows shows no appreciable variation from north to south.

Turning to the A-K series, it will be seen that B, C, D and G are represented at least once in every row, so that in their case the variation in the soil condition which manifested itself is, in its effect on crop, neutralised.

In respect to A, E and F we are on less sure ground, but as will be seen later, it is possible to arrive at results in respect to them which would appear to be almost equally satisfactory.

The first problem was to find the standard deviation of differences. As B, C, D and G occur in all rows, the pairs BC, BD, Bb, CD, Cb, CG were taken first. Whenever B, C or D was duplicated in a given row, both possible pairs were taken, but throughout, only pairs in the same row were considered. To this group of thirty-five pairs was added fifteen composed of the pairs EB, EC, and ED, each occurring four times, and EG which occurs three times. Thus ten pairs of varieties gave fifty pairs of plots, each pair in the same row.

The standard deviation thus obtained is 4.10 lbs., which allows of a probable error for one pair of 2.77, or for four pairs of 1.38.

There is next the difficulty of obtaining comparable mean yields for each variety. The data for B and C are:

Row	B	C
1	19.5	21.5, 28.25
2	26	31.5
3	32.5, 39	35.5
4	56	51

If we average these results as they stand, C will be given two plots in the poor row 1, and B two plots in the good row 3. The average of B will thereby tend to be made greater than the average of C. This will not do. Where any variety had more than one plot in a row, the figures for these plots were therefore replaced by their mean, and the average calculated from the four resulting figures. In this way we get the comparable or standardised yields for B, C, D and G:

Standardised yield.	
B	34.3
C	35.7
D	41.6
G	40.6

The yields of B, C and D are in the same order as size of set, and the difference (7.3 lbs.) between the greatest and least is over five times the probable error of the difference (1.4)—taking this probable error as based in effect on only four pairs of plots.

But there remain A, E and F of the variety used for testing the effect of size of set. A has plots only in rows 1, 3 and 4: hence the averages obtained in the same way as above for A and for the plots in the same rows of B, C and D are not comparable with the standardised figures above; and we want a comparable figure.

This was obtained in the following way: on the three pairs of plots available, the total yield of A was 76.5, and of B 111.2. The standardised yield of B is 34.3. An estimated standardised yield of A may therefore be put at $76.5 \times 34.3 \div 111.2$ or 23.6. From the similar figures for C, D and G we obtain estimates of 24.5, 24.3 and 24.4. These figures are all fairly close together, and we may take their mean, 24.2, as a fairly close estimate of the standardised yield of A. Standardised estimates for E and F were similarly obtained, viz. E, 47.5, and F, 67.3.

These figures increase without a break from the smallest sets A, to the largest sets F, so there can be no doubt about the result. For B, C and D, as already stated, the probable error of a difference is about 1.4 lbs. For A, E and F, owing to the method of estimation, we cannot state a precise probable error. But if the probable error of the difference

188 *Influence of Seed Weight, etc., on the Potato Crop*

between A and F be as large as 3 lbs., the actual difference between their yields is over fourteen times this.

The standardised mean yields for each of the seed classes A to G can now be given:

A	24.2	E	17.5
B	34.3	F	67.3
C	35.7	G	40.6
D	44.6		

and are shown diagrammatically in Fig. 2, from which it is clear that the crop increases directly with the weight of the tuber set. If the mean is taken of the values of the series A-F, we obtain the figure 44.8 for the mean yield of the six seed classes, which corresponds very closely with 40.6, the standardised yield for G, the seed class where tubers are planted without any conscious selection. This close approximation is of particular interest, for it not only confirms the general correctness of the calculations, but it shows that the choice of the six seed classes A-F probably covers all the chief possibilities illustrating the variation of yield arising from seed weight differences.

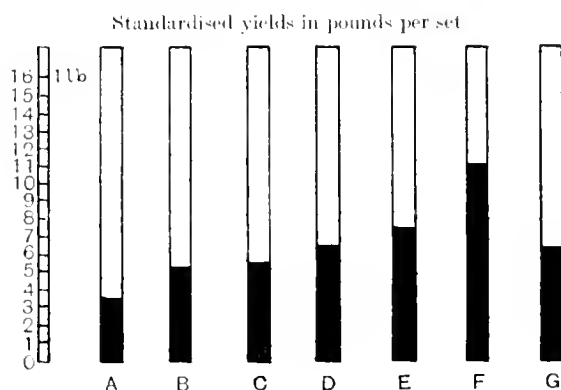


Fig. 2.

Middleton¹ also found a positive correlation between yield and size of set but only dealt with three sizes.

The 1921 results differ in one respect from those of 1920, where the 2 ozs. set produced the maximum yield, whereas in this year the yield increases *pari passu* with the weight of the set. It may well be that the latter result is the generally correct one, but the possibility must not be

¹ Middleton, T. H., *Guide to Experiments conducted at Burgoyne's Farm, etc., Camb. Univ. Dept. of Agriculture, 1907.*

overlooked that in a normal season the sets weighing 2 ozs. or thereabouts might again prove to be the best, and that the advantage accruing to heavier sets in 1921 is due indirectly to the great drought, when the absence of moisture in the soil gave a fictitious value to the heavier tuber-sets because of the greater quantity of moisture contained in and about their relatively larger bulk.

The lots H, J and K, representing the tuber-sets classes with outgrowths, produced crops of 50, 64.6 and 50 lbs. respectively. In the 1920 trials the outgrowth tuber-sets were whole and weighed 6 ozs. each, and produced a crop 25 per cent. bigger than any other class of seed. In this year's experiment there were no large tubers with outgrowths. Indeed, the latter, on any size tubers were rare.

The outstanding feature is the high yield of class J, the cut sets with outgrowths of average weight 2 ozs. This exceeds the yield of H or K by 14.6, which is five times the probable error for a single pair, and although it is true that the probable error for a plot of 65 tuber-sets is greater than for one of 100, yet a difference as great as here observed cannot be regarded as other than very significant.

Assuming, therefore, as we may, that the excess of yield of the J class over that of H or K is to be referred to the difference of kind in the seed set, it becomes of interest to consider what this difference really consists in.

In class J, the only buds left are those eyes on the secondary outgrowth, whilst there are 2 ozs. of flesh for their shoots to feed on in their early stage. In class H we have whole tubers of the same weight as the J class, but here the outgrowth is only a part, and that a minor one, of the whole tuber, and the shoots arising from it have to compete for nourishment with those arising from the other eyes of the tuber, and hence have not the initial advantage which the J class enjoys.

Class K consists of isolated outgrowths with but small quantities of tuber material to feed on and is, in a season such as that of 1921, at a distinct disadvantage.

Class I consists of the rose or crown ends of the tubers from which the cut sets of J and K have been removed. Their average weight was 1.66 ozs. There is no reason to expect that these sets should possess any greater advantage than any other tuber-set of the same size which was devoid of outgrowth. Indeed, in respect to weight of tuber-set the yield that might be expected would be something between that given by classes B and C in the same row, say 52 lbs. However, the realised yield of 40 lbs. is sufficiently widely removed from this figure to suggest a difference of

190 *Influence of Seed Weight, etc., on the Potato Crop*

significance, and one is tempted to see in this reduced yield a confirmation of the conclusions reached by Middleton¹ that in general, cut sets were inferior in cropping capacity to whole tubers.

As in 1920, so again in 1921, the realised crops from each class were analysed in the following manner:

A 10 lb. sample was weighed out on a spring balance and was then sorted into the following classes:

Tubers weighing 2 to the lb.				Tubers weighing 11 to the lb.			
..	..	3	16	..
..	..	4	18	..
..	..	5	20	..
..	..	6	24	..
..	..	7	30	..
..	..	8	36	..
..	..	10	under this weight		
..	..	12	..				

It was found that when this subdivision was made and the individual groups summated, they always came to a figure within a little of 10½ lbs., the excess being due to error of the balance in weighing small lots. It is for this reason that the relative proportion of the different classes shown in Figs. 3 and 4 is calculated to 10½ lbs. It should be realised that in each of these classes into which the crop has been subdivided, the tubers are practically of exactly equal weight and size. The actual figures are given in Schedule II.

Although in all the samples, division into all the 17 classes was carried out—as far as the material in each case allowed—it was found that for practical purposes it was better to re-group the findings into four classes:

1. Tubers of 3.33 ozs. and over.
2. .. over 2 ozs. and under 3.33 ozs.
3. .. 1 oz. .. 2 ozs.
4. .. under 1 oz.

As regards classes B, C, D and G, the mean value of each weight group in the crop was readily determined, always, however, taking the mean of the determination where two examples of a class occurred in one and the same of the four rows in the experimental plot.

In regard to A, E and F, the same method in determining the mean

¹ Middleton, T. H. *Guide to Experiments conducted at Burgoyne's Farm, etc., Camb. Univ. Dept. of Agriculture, 1907.*

value of each weight group was applied as was used to determine their standardised mean yields.

The results are shown in Fig. 3. Concentrating on the production of heavy ware (Class 1 of the weight series rendered solid black in the figure) it will be seen that, except for a very slight increase, viz. .5 per cent., of this class in the B series over the A, there is a steady decrease of heavy ware with every increase of weight of the seed.

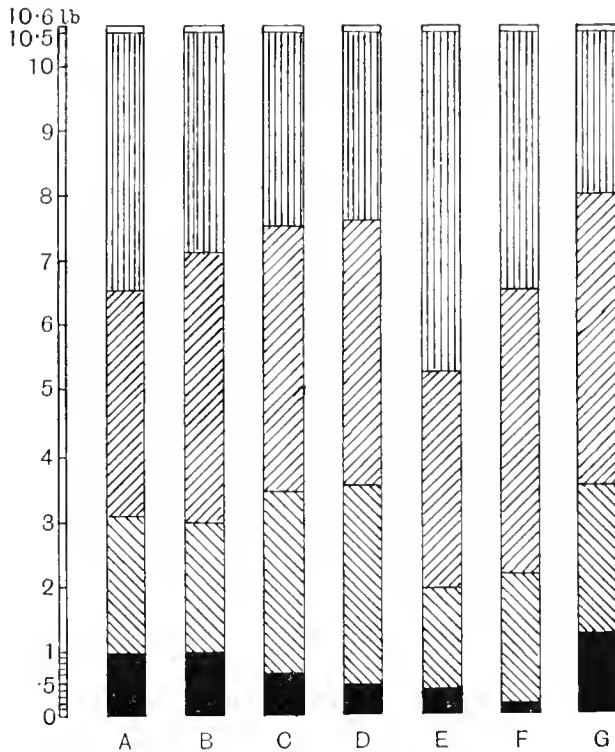


Fig. 3.

A. Sets weighing .6 oz. each.

B. „ „ 1.33 ozs. „

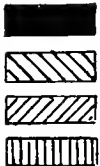
C. „ „ 2.0 ozs. „

D. „ „ 2.66 ozs. „

E. Sets weighing 4.0 ozs. each.

F. „ „ 5.33 ozs. „

G. „ unselected.



Represents the weight of tubers of 3.33 ozs. and over in every 10½ lb. sample.

„ „ „ 2.0 ozs. „ „ „

„ „ „ 1 oz. „ „ „

„ „ „ less than 1 oz. in wt. „ „

192 *Influence of Seed Weight, etc. on the Potato Crop*

Turning to the production of the lighter tubers in the yields, two facts emerge. In the series A-D, the quantity of "chats" decreases in direct ratio with the weight of the set, and therefore in the same ratio as that of the "ware" in the same yield. On the other hand, in the E and F tuber-set series the amount of "chats" is much in excess of that found in any of the A-D groups. So that it would appear from these results to be very clearly demonstrated that heavy sets, weighing 4 ozs. and over, not only give greatly reduced quantities of useful heavy ware, but also return in their produce an altogether excessive proportion of useless chats in comparison with the yields rendered by tuber-sets of smaller size. This result is entirely in accord with that obtained in the favourable season of 1920, when the sets weighing 4 ozs. and over produced roughly twice as many chats, and one-third or more less ware, than the sets of lighter weight.

A similar analysis of the yields was made of the tuber-sets groups H, I, J and K, and the results are shown in Fig. 1, where they are placed in comparison with all the other groups represented in the same portion of the experimental plot. In this series the outstanding fact is the enormous proportion, viz. 40 per cent., of heavy ware produced by the K series. If reference be made to Fig. 1 it will be seen that series K and F were grown in the same line in the same portion of the experimental plot, and so may be fairly compared. The F series, however, are tuber-sets of 5.33 ozs., whilst the K are outgrowths of 1.25 ozs. in weight, and the great disproportion, between 5 per cent. and 40 per cent. of the whole sample, the quantities of heavy ware produced by them respectively, is confirmatory evidence of the previous deduction, that the lighter the weight of the tuber-set, the greater the proportion of heavy ware produced.

This relation of size of set to quantity of heavy ware was observed by Sir Thomas Middleton¹, but his results were unknown to the author till after this paper was written.

No explanation is advanced either of this relation or of that between the weight of set and the weight of the total crop, but it is permissible to suggest that both phenomena may be related to the lesser maturity of small tubers.

In each of the analyses of crops made,—and many were duplicated—record was kept of the presence of secondary outgrowths on the tubers. Doubtless owing to the fact that this particular piece of land never

¹ Middleton, T. H., "Potato Experiments at Burgoyne's Farm, Impington, Cambs.," *The Potato Year Book*, 1907.

received any of the late rains enjoyed by other parts, there were but very few, and they of very small size. It was found that their presence was not correlated with any feature such as size of tuber, nor were they any more frequent in the group H, I, J or K than in those of the series A-F. As in 1920, no evidence is forthcoming that such growths are conveyed by tubers from one generation to another.

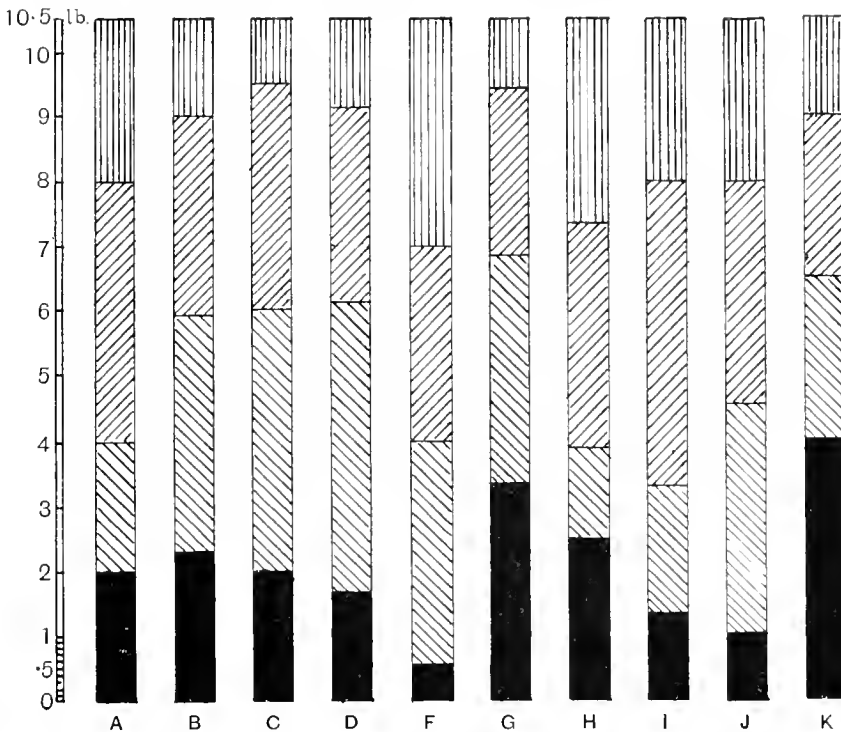


Fig. 4.

A—G. As before.

H. Whole tubers with outgrowths, 2 ozs. each.

I. Crown ends from tubers with outgrowths, 1.6 ozs. each.

J. Cut sets with outgrowths, 2 ozs. each.

K. Cut sets with outgrowths, 1.25 ozs. each.



Represents the weight of tubers of 3.33 ozs. and over in every 10½ lb. sample					
"	"	"	2 ozs.	"	"
"	"	"	1 oz.	"	"
"	"	"	less than 1 oz. in wt.	"	"

194 *Influence of Seed Weight, etc. on the Potato Crop*

The results attained in 1921, which so closely bear out those suggested by the experiment of 1920, may be briefly summarised:

1. The total yield varies directly with the weight of the tuber-set.
2. That small sets under 1 oz. in weight, although giving a great return in proportion to their weight, and a high proportion of heavy ware, are uneconomical.
3. That taking into consideration the total weight of seed used, the proportion of heavy ware produced and the total yield, sets of 2 ozs. in weight are the most remunerative.
4. Cut sets consisting of secondary outgrowths weighing 2 ozs., and whole sets with similar outgrowths of the same weight to a lesser extent, produce considerably heavier crops than any other type of set, and at the same time produce a high quantity of heavy ware.
5. There is an inverse ratio between the size of the seed set and the percentage of heavy ware in the resulting crop.
6. The productivity of secondary growth, as well as the high proportion of heavy ware, yielded by small tuber-sets, may be correlated with immaturity of the seed tuber.
7. There is no correlation between the presence of secondary growth in the seed set and the existence of the same in the resultant crop.

Schedule 1. *Showing the weights of crops in pounds of each seed class in the experimental area, including the Guard Rows.*

Guard rows									Guard rows	
4	3	A	B	C	D	E	G	C	2	1
9.5	11.5	12.5	19.5	21.5	26.5	35.5	30.5	28.25	10.5	10.5
		D	E	F	C	B	D	G		
10	16.5	32.25	37	49.25	31.5	26	39	35.25	16	20
		B	C	A	G	D	E	B		
11.9	17.4	32.5	35.5	27	39.25	48.25	48.25	39	21	19.5
		C	G	H ¹	D	B	G	A ⁴		
16	24	51	56.5	7.5	56	56	58	18.5	27	21.5
				I ²				F ⁵		
				8				10.5		
				J ³				K ⁶		
				42				20		

¹ H, 15 sets planted.

⁴ A, 50 sets planted.

² I, 20 „

⁵ F, 10 „

³ J, 65 „

⁶ K, 40 „

Schedule II. *Analysis of crops of each of the seed classes as they occur in the experimental area, showing the amount in pounds in each weight grade excepting the 'chats' under 1 oz. out of sample weighing 10.5 lbs.*

Tubers weighing over 3.3 ozs.

A	B	C	D	E	G	C
0	0	0	0	0	0	0
D	E	F	C	B	D	G
0	0	0	.5	0	0	0
B	C	A	G	D	E	B
2	.5	2	1.25	.5	.5	1
C	G	H	D	B	G	A
2	3.2	2.5	1.6	2.33	3.5	2
		I				F
		1.3				.5
		J				K
		1				1

Tubers weighing over 2 ozs.

A	B	C	D	E	G	C
2	1	2.5	1.5	1.5	1.5	2.5
D	E	F	C	B	D	G
2.6	1	1.5	1.75	1.5	3	2.37
B	C	A	G	D	E	B
4	3.5	1.5	3.25	3.75	2	3
C	G	H	D	B	G	A
6	6.6	3.9	6.1	5.83	7	4
		I				F
		3.3				4
		J				K
		4.5				6

Tubers weighing over 1 oz.

A	B	C	D	E	G	C
4.5	5.5	6	5.5	5	5.5	7
D	E	F	C	B	D	G
7.6	5.5	6	6.5	6	8	6.75
B	C	A	G	D	E	B
7.75	7.5	7	8.25	7.75	5.5	7.5
C	G	H	D	B	G	A
9.5	9.1	7.3	9	9	10	8
		I				F
		8				7
		J				K
		8				9

DESCRIPTION OF FIGURES.

- Fig. 1. Shows the plots as actually laid out. In the lowest row it should be understood that Lots H, I and J formed one row, and A, F and K another. The blackened portion represents the yield per set in pounds, in each plot.
- Fig. 2. Represents the standardised yields per set in each of the classes of seed weight A-G.
- Fig. 3. The crops from A-G are analysed as to the weight groups into which their constituent tubers fall. The figure represents the standardised mean of the various groups in each of the classes.
- Fig. 4. The analysis of crops of all seed classes in the fourth or western section of the experimental plot showing the relation of the special classes H, I, J and K to the normal.

The writer has great pleasure in acknowledging his deep obligation to Mr Udhay Yule, C.B.E., M.A., F.R.S., who most kindly worked out and elucidated the statistical data in this paper.

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AN INVESTIGATION UPON CERTAIN METRICAL ATTRIBUTES OF WHEAT PLANTS.

By F. L. ENGLEDOW, M.A.

Plant Breeding Institute, School of Agriculture, Cambridge.

AND J. P. SHELTON,

Farrer Memorial Scholar, Sydney.

CONTENTS.

	PAGE
§ I. Introduction	197
§ II. Material and Method	199
§ III. The Glume-Length : Rachis-Length Ratio	200
§ IV. Concerning the Inter-relationship of the Tillers of a Plant in regard to certain Measurable Characters	202
§ V. The Relation of Weight of Seed Sown to the Resulting Plant	203
Conclusions	204
Bibliography	205

§ I. INTRODUCTION.

IN dealing with the Inheritance of Glume-Length [Engledow(1)] there were encountered certain problems relating to metrical characters. As it appeared that these problems must attach to all genetic work upon a metrical basis, they were made the subject of a separate investigation. A simple account of some of the difficulties experienced in connection with Glume-Length Inheritance will serve to formulate the problems.

It is necessary first to set forth the reasons which led to a genetic investigation upon glume-length. To the glume itself no intrinsic interest attached. The prime motive was to forge some sharper weapon than eye-judgment for the separation of "genetic types" in F_2 's etc., and for more critical study of segregation. Rigid measurement, and classification solely by measurement, might, it was felt, provide such a weapon. Alike to eye-judgment and to measurement, "fluctuation" was certain to be an obstacle; but both observation and inference united to suggest that the length of the glume of the wheat plant was less liable to fluctuation than were most of its other observable attributes. There was the additional advantage that glume-length could be measured accurately and

with facility. These two reasons, then, led to its adoption. Overlap of distributions is the form in which the difficulty of fluctuation makes itself felt. To counter overlap, two widely differing parent forms were selected, viz. the *T. durum* known as Kubanka (mean glume-length in England ± 10.5 mm.), and the distinctive *T. polonicum* or Polish Wheat (mean glume-length in England ± 30.5 mm.). Greatly as these forms diverged in glume-length, their distributions nevertheless showed a slight overlap. In the F_2 , the parental types reappeared in a "shifted" form. With them, and intermediate between them, came a heterozygote. Consequently the glume-length distribution of the whole F_2 took a trimodal form which, defying analysis on the basis of rigid measurement, enforced a reversion to eye-judgment. It was quite obvious that the wide "fluctuation" in glume-length was due to the occurrence in the parental and segregate populations of a certain number of very poorly grown plants. To reject such plants would have been disastrous and to include them was to confuse distributions and inhibit analysis. Plants of this kind were poor in every way and, roughly speaking, the less the stature of a plant, the shorter were its leaves, its ears, its glumes, etc. This very patent fact suggested that instead of making, for every plant, an absolute measurement, there should be made some form of "compensated" measurement. In short, every plant should be "handicapped." "Length-of-rachis" was selected as the basis of the "handicap." The working hypothesis was that a big, thriving, plant had long ears (*i.e.* great rachis length) on which were borne proportionately long glumes. Per contra, small plants would be small "all round" and it seemed not unlikely that the ratio length of glume : length of rachis would exhibit a smaller plant-to-plant fluctuation than would absolute glume-length. To test the constancy of this ratio, then, became the first object of investigation.

A phenomenon designated by the term "shift" was observed in the F_2 of the Polish \times Kubanka cross. It consisted in the appearance in F_2 of two groups of plants, each in number about a quarter of the whole F_2 population, and having respectively a complete eye-resemblance to the parental (F_0) forms. In mean glume-length, however, the F_2 Kubanka type slightly exceeded the F_0 , while the F_2 Polish type was 25 per cent. shorter than the F_0 . Explanations of "shift" could be based upon "modification by crossing" [cf. Ruggles-Gates(3)] or upon "minor multiplying factors," but yet another explanation seemed possible. The embryos and endosperms from which grew the F_2 Polish-type plants, were nourished by F_1 (*i.e.* heterozygous and intermediate) plants.

Upbringing by such a "foster mother" might have some predetermining influence and might be the cause of "shift" [for a fuller consideration see Engledow(1), pp. 127-8]. This explanation is based upon a belief that there is a fairly close relationship between the weight, composition, etc. of the mother seed, and the attributes (glume-length included) of the resulting plant. To test this relationship became a second object of investigation.

Many morphological and economic attributes of plants may be metrically represented in a number of different ways. Cereal plants illustrate this point. As generally grown a plant has a main axis and a number of axillary shoots or tillers. To obtain an expression of the glume-length, rachis-length, ratio of grain to straw etc. for a population of any variety, it is possible to confine observation to one ear-bearing stalk per plant. The largest, the first formed, or a random ear may be chosen; or every ear of the plant may be included and the observations for the whole plant be averaged. Labour is minimised if only one ear per plant be observed but the available number of observations is increased by the inclusion of every ear. Differing sets of circumstances have led sometimes to the one practice and sometimes to the other and it is clear that the justification of each in its own circumstances, must rest upon the relationships which prevail among the tillers of the individual plant. These relationships formed the third part of the enquiry.

§ II. MATERIAL AND METHOD.

The strains of Polish and Kusbanka were the ones used in the glume-length investigation. They are both old and carefully kept pure lines. The seed for each variety was obtained from forty plants of the 1919 harvest. The main ear of every one of these plants was measured for rachis-length and glume-length and all of the grains were weighed and separately labelled. Sowing (in 1920) was completed in one day and both germination and growth were good and of as great uniformity as is usually attainable under the conditions of careful experiment. At harvest (1920), every ear of every plant was separately collected, and later on its rachis-length and glume-length were determined. Previously it had been usual to measure only one glume per ear [see Engledow(1), pp. 111-2], but for this investigation both glumes of the "median" spikelet of each side of the ear (*i.e.* four glumes per ear) were measured. The average of these four measurements is, hereinafter, referred to as "glume-length."

§ III. THE GLUME-LENGTH : RACHIS-LENGTH RATIO.

As a preliminary to further work, the correlation between glume-length and rachis-length was evaluated from data obtained from the 1911 crop of the two pure lines. There were 150 plants of each pure line and the coefficients of correlation (r) were:

$$\begin{aligned}\text{Polish} & \quad 0.295 \quad 0.050, \\ \text{Kubanka} & = \quad 0.469 \quad 0.043.\end{aligned}$$

It seemed not improbable that the lowness of the correlations was due to the fact that the experimental plants were not alike in respect of the total number of ears (or tillers) produced per plant. Consequently the 1920 plants—the ones observed in the main investigation—were classified as “one-ear,” “two-ear,” etc. plants and the correlation between glume-length and rachis-length was evaluated for the separate classes. In the case of every class, the main ear only of every plant was dealt with in the correlations. Table I contains the correlation coefficients (r):

Table I*. *Coefficients of Correlation between Glume-Length and Rachis-Length for one-ear, two-ear, and three-ear Polish and Kubanka plants.*
(Only the main ear of the plant was observed.)

Variety	No. of ears per plant	No. of plants	r	
Polish	1	316	0.918	0.006
Polish	2	140	0.768	0.026
Polish	3	38	0.852	0.030
Kubanka	1	205	0.751	0.020
Kubanka	2	107	0.717	0.032
Kubanka	3	55	0.494	0.069

* All the correlations in the table are positive. The unit of measurement throughout is 1 mm.

The values of the correlations are high, both absolutely and in relation to their own probable errors but even a correlation of unity between two variables does not imply a constant ratio between them unless the regression lines pass through the origin (0, 0). This fact was well illustrated by the results obtained when the ratio glume-length/rachis-length was evaluated for individual plants. The ratio fluctuated as wildly as did the glume-length and to show this it is necessary to give no more than the coefficients of variation (viz. $v = 100\sigma/M$, where σ = standard deviation and M = mean). These are given in Table II.

Table II. *Coefficients of Variation for Glume-Length, Rachis-Length and the Ratio of these two quantities in Polish and Kubanka wheats.*

Variety and no. of plants	No. of ears per plant	Coefficient of variation of		
		Glume-length	Rachis-length	Ratio
Polish (316)	1	17.13 \pm 0.47	25.77 \pm 0.74	15.47 \pm 0.43
Polish (110)	2	8.56 \pm 0.39	13.83 \pm 0.64	10.83 \pm 0.49
Kubanka (205)	1	10.18 \pm 0.34	20.43 \pm 0.71	18.29 \pm 0.63
Kubanka (107)	2	8.46 \pm 0.39	16.88 \pm 0.80	12.58 \pm 0.59

It is therefore to be concluded that this particular "compensated" ratio possesses no value either to the geneticist for a critical study of variation or to the plant breeder for the discrimination of closely resembling agricultural strains.

Despite this record of failure, it is felt that "compensated" observations (*i.e.* some form of ratio) offer still the only alternative to "absolutely (unattainably?) uniform conditions of growth" as a means of removing the masking effects of "fluctuation." It is not to be expected that the random choice of two characters—*e.g.* leaf-width and grain-length—will serve as a basis for compensation or handicap and provide a constant ratio. Manifestly, in seeking constancy, one should endeavour to find two "lengths" (or other attributes) which are determined by the same causes and during the same time-period. In glume- and rachis-lengths, it would seem, these requirements are as likely to meet fulfilment as in any pair of attributes of the wheat plant to which one could point. This granted, there follows the regrettable but not surprising conclusion that "lengths" are such vague expressions of the real "nature" of a plant variety, of its physiological activities, as to be of little value in attempts to determine accurately its modes of inheritance.

The employment of "ratios" by geneticists has usually been dictated not so much by a desire to achieve "compensation" as by the necessity of devising representations for elusive attributes like leaf-shape, etc. The work of Martin-Leake⁽⁵⁾ upon Cotton, of Balls⁽⁶⁾ upon the same plant, and of Groth⁽⁷⁾ upon the Tomato afford examples; but in all these cases fluctuation has played its customary havoc.

In outline, the "handicap" principle for plants is strictly analogous to that followed in flat-racing. Horses are handicapped so that they may afford an exciting "bunch" at the winning-post. It is required to "bunch" metrical observations upon a plant population (*i.e.* to minimise fluctuation) but it seems probable that a really successful "handicap" will have to be a complex one. It will, in fact, have to be applied not

simply to the "end-product" (the mature plant) but to the more important life-stages by which the end-product is determined.

§ IV. THE INTER-RELATIONSHIPS OF THE TILLERS OF A PLANT
IN REGARD TO CERTAIN MEASURABLE CHARACTERS.

Glume-length, rachis-length, and the ratio of these two, were determined for every ear of every plant of Polish and Kubanka (1920 crop). Ear-to-ear correlations (same plant) are given in Table III. It is plain that first and second ears are more closely correlated than first and third; further, correlations for rachis-length are slightly higher than those for glume-length. Of the actual coefficients of correlation it may be said that they are statistically significant but are not very high (their general value is about ± 0.5).

Table III. *Ear-to-ear Correlations on the Individual Plant.*

Variety and no. of plants	<i>r</i> for	Value of <i>r</i> for			Ears per plant
		Glume-length	Rachis-length	Ratio	
Polish (110)	Ears 1 and 2	0.501 \pm 0.050	0.665 \pm 0.036	0.512 \pm 0.047	2
Polish (38)	Ears 1 and 3	0.429 \pm 0.089	0.439 \pm 0.089	0.428 \pm 0.089	3
Kubanka (107)	Ears 1 and 2	0.599 \pm 0.042	0.659 \pm 0.037	0.699 \pm 0.033	2

The first inference from these figures seems to be that, when dealing with the glume or with any similar ear-character, it is essential to confine the observations to one ear per plant. In that, as a very general rule, the ear of the main stalk is formed first and attains the greatest growth, it is the best ear of the plant for observation. This inference from the facts of inter-tiller correlation naturally leads one to ask whether, in furtherance, it would be well to limit the experimental population to plants which all possess the same total number of tillers or which all ripen the same number of ears. Upon this point further evidence is available and it is set forth in Table IV. The facts are quite clear and

Table IV. *Mean Values for the main tillers of plants grouped according to the number of ears per plant.*

Variety and no. of plants	No. of ears per plant	Mean value (main tillers only) of		
		Glume-length	Rachis-length	Ratio
Polish (316)	1	26.5 \pm 0.17	92.1 \pm 0.90	0.298 \pm 0.002
Polish (110)	2	30.6 \pm 0.17	118.4 \pm 1.05	0.262 \pm 0.002
Kubanka (205)	1	10.9 \pm 0.05	58.0 \pm 0.56	0.196 \pm 0.002
Kubanka (107)	2	11.7 \pm 0.06	68.5 \pm 0.75	0.175 \pm 0.001

rather striking. Although only the main tiller was observed in the case of every plant, the one-ear and two-ear plants give very different results.

Both for Polish and Kubanka, glume- and rachis-lengths are greater in the case of two-ear than in the case of one-ear plants; and since the difference is more marked for rachis than for glume, the "ratio" is lower for the two-ear plants.

A difference is, perhaps, to be anticipated for, within limits, the more vigorous the plant the more tillers it produces: and the facts displayed in Table IV make it appear that greater vigour is evinced in "all-round" form—not only more tillers but bigger ones (larger glumes and rachis). This "co-fluctuation" of the attributes rachis-length, glume-length, and number of tillers, encourages one to think that in such attributes is to be found a means of estimating degree of growth and of comparing and contrasting different pure lines. There may still be room for hope but no progress is possible until "fluctuation" has been dealt with and, as far as this case goes, the mathematical handling of attribute measurements (use of ratios, etc.) has been valueless.

It has been inferred that observation should be confined to the main ear of the plant and this inference, if valid, is not without interest from the point of view of yield-investigations. When, in seeking higher-yielding forms, an F_2 is raised from two parental strains, it is almost essential to cast out what are believed to be the "inferior" segregates. Even if there be no casting of F_2 plants, the process must be applied to the resulting F_3 families for otherwise available time and ground-space become inadequate. In actual practice "casting" by eye-judgment has attained some very conspicuous successes but there is now a tendency to try to substitute an accurate "method" in its place. If those attributes which are considered to govern yield behave in a way analogous to that described for glume-length, etc., then the best tiller and not the whole plant should be the basis of estimation. In the field, however, small tillers as well as large go to make a crop, and whatever form of judgment of F_2 plants is evolved, it must of course, in some way, pay due regard to tillering power.

§ V. THE RELATION OF WEIGHT OF SEED SOWN TO THE RESULTING PLANT.

It has been explained in the introduction that there existed a special reason for attempting to determine the influence upon the plant of the weight and composition of the seed from which it grew. Apart from this, in a great mass of literature [for a very full summary see Kidd and West(s)], there is evidence which justifies the supposition that much of the troublesome "fluctuation" is induced by lack of uniformity of seed.

As a contributory test of this point, correlations were evaluated between weight of mother-seed and glume-length, rachis-length, and their ratio in the resulting plants. Preliminary investigation having shown an absence of correlation for a population of plants having different numbers of ears, attention was confined to single-ear plants. Table V contains the results.

Table V. *Correlation between Weight of Mother Seed and the Characters of the Resulting Plant.* (Only one-ear plants are included.)

Variety and no. of plants	Correlation between weight of mother seed and		
	Glume-length	Rachis-length	Ratio
Polish (316)	0.065 ± 0.038	0.043 ± 0.038	0.000 —
Kubanka (205)	0.262 ± 0.044	0.157 ± 0.016	0.003 0.047

The supposed relation between weight of mother-seed and glume-length, etc., is thus emphatically negatived. Mere weight of seed is not—save at the lower extreme—likely to be of great physiological importance and the belief in its importance (vide literature above mentioned) is, perhaps, largely due to the form of experiment often adopted—the removal of a portion of the endosperm, work with non-pure lines, fewness of observations and so on. No embryo during germination uses the whole of the reserve food with which it has been provided and reserves beyond a certain amount must be simply “surplus.” That “quality” of endosperm may be important still remains a possibility and evidence exists to this effect. This possibility is the only remaining basis for the explanation of “shift” of which an account is given in the introduction. With the general principle that good, sound, seed must be sown to reap a good crop, there is common agreement, but beyond this, even in genetic work and in face of the danger of fluctuation, there seems no need to go. The weighing of a vast number of seeds in order that seeds all of the same weight may be sown, seems, from the facts above recorded, not to be worth while.

CONCLUSIONS.

The conclusions which follow hold in strictness—failing further test—only for the year, the locality, and the wheat varieties concerned in the investigation. Since, however, the results are fairly emphatic, they seem likely to prove applicable in principle to other circumstances.

(i) Glume-length and rachis-length in both Polish and Kubanka Wheats are very highly correlated.

(ii) Nevertheless, the ratio of these two quantities has about as big a coefficient of variation as the absolute glume-length.

(iii) Therefore, despite contrary expectations, the ratio appears to be of no special value in investigation.

(iv) Among the tillers of any one plant correlations exist for glume-length, rachis-length, and ratio. Their general value is about $+0.5$ and consequently when dealing with attributes of this kind observation should be confined to the main stalk of every plant.

(v) It is desirable further, to restrict the experimental population to plants all of which produce the same number of tillers.

(vi) Weight of mother-seed, for a reasonably good seed sample, seems not to determine in any observable degree the growth of the resulting plant as judged in general by glume-length, rachis-length, and the ratio of these two.

(vii) The great labour of picking out for sowing a sample of seeds all of one weight in order to reduce "fluctuation" among the resulting plants, seems not likely to be repaid.

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CONTENTS.

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	PAGE
1. MURRAY, ALAN J. The chemical composition of animal bodies. (With 1 Text-figure.)	103
2. TAYLOR, WILLIAM and HUSBAND, ALFRED D. The effect on the percentage composition of the milk of (a) variations in the daily volume and (b) variations in the nature of the diet. (With 4 Text-figures.)	111
3. TOCHER, J. F. The citric solubility of mineral phosphates. (With 8 Diagrams.)	125
4. WOODMAN, HERBERT ERNEST. Comparative determinations of the digestibility and metabolisable energy of green oats and tares, oat and tare hay and oat and tare silage	144
5. WHITTLES, C. L. A note on the classification of soils on the basis of mechanical analyses. (With 11 Text-figures.)	166
6. SALAMAN, REDCLIFFE N. The influence of size and character of seed on the yield of potatoes. (With 4 Text-figures.)	182
7. ENGLEADOW, F. L. and SHELTON, J. P. An investigation upon certain metrical attributes of wheat plants	197

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SOME INVESTIGATIONS ON THE ELECTRICAL
METHOD OF SOIL MOISTURE DETERMINATION.

BY THOMAS DEIGHTON, M.A., B.Sc.

*(School of Agriculture, Cambridge.)**(With Six Text-Figures.)*

THE investigations described in the following pages had their origin in a desire to find some method of moisture determination in the soil which would not require the taking of samples, with a view to some further experiments on the cultivation of the soil in root-crop production.

Tentative work on these lines was commenced in America in 1887 by Professor Milton Whitney⁽¹⁾ who employed an earth battery consisting of alternate copper and zinc plates buried in the soil, connected through a galvanometer. Later in *Bull.* No. 6, "Division of Soils," U.S. Dept. Agriculture the same author further develops this method of moisture determination.

In 1897 M. Whitney and T. H. Means⁽²⁾ published an account of some experiments in which they determined the specific resistance of various soils, making a correction for packing error, and showed that this varied considerably for different soils. The authors plotted the resistance against the moisture in various soils and found that the curves obtained "agree in the main with an hyperbolic curve except that they are more or less rotated," and they discovered that "it requires from 1 to 3 per cent. more water to produce the same change in the resistance of some soils than in others." To this paper is appended a table for the reduction of soil resistances to a standard temperature. In the experiments a compensating temperature cell was used.

The following year F. D. Gardner⁽³⁾ dealt with the use of underground cables on ploughed land and gave results of an extended series of measurements of soil moisture by the electrical method compared with determinations by drying on the same plots. The results, though considered satisfactory by the author, show a mean difference of about 2 per cent. in the moisture content between the two methods of working. The maximum difference was as high as 1.3 per cent.

208 *The Electrical Method of Soil Moisture Determination*

In 1899 L. J. Briggs⁽⁴⁾ described improved instruments and electrodes and introduced a condenser in parallel with that arm of the bridge adjacent to the soil resistance, which would throw the small capacity found in the soil and the condenser capacity on opposite sides of the bridge with respect to the telephone receiver.

I have found a reference to some work by R. O. E. Davis (*Trans. Amer. Electrochem. Soc.*, **17** (1910), 391-403), in which he is said to have found the resistance of soils within the limits 10-20 per cent. of moisture to be inversely as the moisture content. Unfortunately the original of this paper is not to be obtained here, but the result thus stated is at variance with Whitney's results which experiments to be described in this paper fully confirm.

The electrical method has never been popular on this side of the Atlantic for several reasons, notably the discrepancy in the results attained by Gardner using the two methods in a parallel series of experiments and the failure of the investigators to deal satisfactorily with the question of movement of salts in the soil.

PRELIMINARY EXPERIMENTS.

Apparatus. The experiments which follow were made on a small plot of ground on the south side of the School of Agriculture at Cambridge. It had been dug over a short time previously and was not ideal for the purpose as a thin layer of builder's refuse from the building of the school rendered it less homogeneous than cultivated farm land. It had, however, the merit of being conveniently situated with regard to the laboratory, and the results obtained upon it appear to justify the conclusion that no great error was introduced by this lack of uniform texture.

For the measurement of the resistance an ordinary post-office pattern Wheatstone's bridge was employed, with an induction coil from which the condenser had been removed to avoid any possibility of polarisation of the electrodes, and a telephone receiver. As a source of energy two small storage cells were used. At first a large coil giving a frequency of something under 30 per second was put in, but this was afterwards changed for a smaller one with a frequency rather over 100 per second, as it was found much easier to estimate the minimum of sound with this. The condenser in parallel with the bridge arm used by Briggs was dispensed with, being found unnecessary for the attainment of the degree of accuracy aimed at.

The electrodes employed were formed from cored electric arc light carbons 9" long and $\frac{3}{4}$ " diameter. The tapered ends of these were ground

down to form roughly a paraboloid of revolution, a shape found most satisfactory by Briggs (*l.c.*) for maintaining contact with the soil. The core was then drilled out for about an inch from the end and the cavity filled with melted caoutchouc and sealing wax, a mixture which maintained its position in the cavity and rendered good service for insulating the end of the core throughout the experiments. The opposite end was then scored and an inch of the core drilled out. A thread was tapped on to the inside of the cavity and a copper wire sealed in with fusible alloy. The top was then covered with sealing wax. The whole of the outside of the electrode, except a band one inch wide round the paraboloidal surface at the bottom, which was to act as the electrode surface, was then insulated by painting with two coats of "Duoprene" and thoroughly dried for two or three days at about 50° C. As the experiments progressed this insulation was found unsatisfactory and in cases where it was necessary to leave the electrodes a considerable time buried another type was adopted which will be described in a later paper.

There appears to be a theoretical objection to the use of metal electrodes of any kind in direct contact with the soil which has not hitherto been noticed by workers in this field; namely that polarisation is very likely to take place even when an alternating current is employed unless the frequency is very high, since the free metallic ions are not completely returned to the electrode on reversal; and in some cases rapidly form double salts which are not decomposed on reversal. In these cases polarisation may occur even at frequencies of 40,000 per min. Owing to its ability to absorb gases a carbon electrode is self-depolarising to a sufficient extent to nullify the first effect and the second does not arise. The phenomenon mentioned is well known to electro-chemists(5).

Local Variations in Resistance. It was desirable before proceeding further to find whether the resistance was the same under like conditions in different parts of the plot. The method of experiment was simply to push two such electrodes as have been described into the soil of the plot to the desired depth at a definite distance apart; a preliminary test having shown that the contact error in this, while not negligible, did not seriously affect the results. The same pair of electrodes was then moved to another part of the plot and placed at the same depth and the same distance apart. The results for a depth of 7" at 3" interval are shown in Fig. 1, which may be taken to represent the plot, which was about 22' \times 30' in extent with the observations entered in the place in which they were taken. Subsidiary experiments proved that the observations were sufficiently close together to justify the insertion of the

210 *The Electrical Method of Soil Moisture Determination*

lines of equal resistance as shown. These results are quite in accordance with the observations of Gardner (*l.c.*), who in attempting to standardise a pair of electrodes on an experimental plot found, by actually drying samples, that the moisture content at any depth might vary over a small area by as much as $7\frac{1}{2}$ per cent. Although moisture contents were not determined in the present case it will be seen from the resistance-moisture observations given later in this paper (Table VII) that such variations are sufficient to account for the different resistances observed, although it should be remembered that other factors may also be operative, such as variations in the soluble salt content—especially of nitrates—from point to point in the plot.

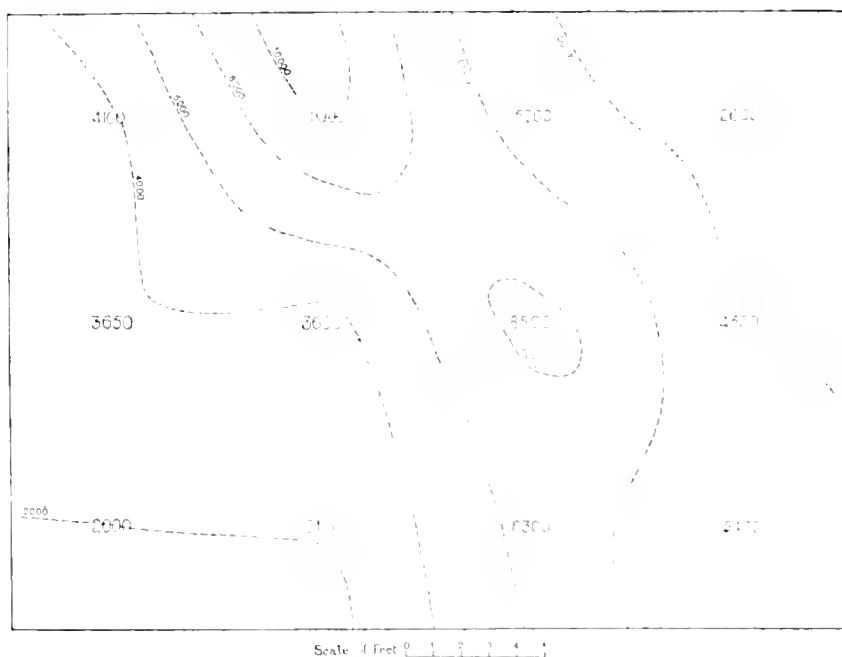


Fig. 1. Local variations of resistance on plot at 7" depth.

The Effect of Distance apart of the Electrodes. Gardner (*l.c.*) made numerous experiments on this matter by burying two large electrodes 15' apart, and a series of smaller ones at distances from $\frac{3}{4}$ " to 18" apart, 15' from each of the larger ones. He then took the resistance between the large pair and between the smaller ones, singly, and each of the larger ones. He adds "From these measurements it was a simple matter to calculate the resistance due to each of the individual electrodes at

a distance of 15 or more feet. By adding the values of any two of the small electrodes thus found, the resistance is obtained which they would have if 15 feet apart." A table is given showing the actual resistance between the small electrodes at their actual distance apart and the calculated resistance between them when 15' apart. He concludes that "when the electrodes are 15 ft. apart 98 per cent. of the resistance is encountered within 9 inches of the surface of each electrode and the intervening $13\frac{1}{2}$ ft. of soil causes only 2 per cent. of the total resistance." Briggs (*loc.*) states that the resistance is practically confined to volumes of soil not exceeding 6" in diameter with the electrodes as centres. If this be so, there should be no important variation on increasing the distance apart beyond 6"; but both Gardner's results quoted below (Table I), and those of the present author on a more extended scale, show a quite appreciable change beyond this point.

Table I. *Excerpt from Gardner's table.*

Distance apart	Resistance between standard electrodes*
5"	928
8"	934
12"	968
18"	979

* Gardner calls this column (the numbers being given as percentages 92.8 etc.), "Relation of observed to calculated resistance." By making the resistance 1000 ohms at 15' the standard and reducing the other resistances to this the same figures are obviously obtained.

Gardner's experiments were all made at one depth and the author therefore carried out a series of measurements of the effect of distance apart of the electrodes on the resistance of the soil at varying depths,

Table II. *Effect of Distance Apart of Electrodes on Resistance at various Depths.*

Distance apart of electrodes	Resistances, in thousands of ohms at						
	1" depth	2" depth	3" depth	4" depth	5" depth	6" depth	7" depth
2"	100	92	33	15.7	11.0	8.7	6.2
3"	60	27	17.0	14.0	11.9	11.9	8.5
4"	75	34.5	15.5	10.4	8.0	6.4	5.2
6"	85	25.5	14.0	9.4	7.0	5.3	4.0
9"	51	22.0	11.3	7.2	5.9	4.9	4.4
12"	45	15.5	9.1	7.0	5.8	5.0	4.6
18"	80	22	10.9	7.5	5.5	4.5	3.8
27"	70	20.9	13.9	8.2	6.5	5.2	4.0

the results of which are given in Table II. There appears to be a tendency for the resistance to fall to a minimum when the electrodes are about

212 *The Electrical Method of Soil Moisture Determination*

12" apart at shallow depths while in the case of depths about 6" the minimum is found when a distance apart of about 18" is reached.

The Effect of Depth. The following experiment was made to determine the effect of depth unhampered by other considerations. A very dry soil was sifted through a 3 mm. sieve to extract stones and packed as uniformly as possible into a pot. Two electrodes 1" apart were then sunk to different depths in it and the resistance measured. It was thus shown that the resistance between them was approximately halved by sinking to a depth of 1". A repetition of the experiment after saturating the soil gave a result quite in accordance since in this case there must have been some moisture gradient due to the action of gravity on the water in the pores of the soil. The observations are shown in Table III.

Table III. *Effect of Depth in Uniform Soil.*

Depth of centre of electrode below soil surface	Resistance in ohms	
	Dry	Saturated
1"	125,000	550
2	102,000	460
3	72,000	260
4	66,000	200

Electrode Values. A further series of experiments was undertaken to determine whether it was really possible to assign values to the electrodes on a system similar to that employed by Gardner. For this purpose equal sized electrodes were set out at the four corners of a six-inch square and at a depth of 6". The distance apart agrees with Briggs' figure for the volume of soil concerned in the resistance measurements. Observations were then taken in all possible ways between them. In Table IV the results are shown. In the last two columns we have the resistances observed and parallel with these the resistances as calculated from the first four observed resistances by Gardner's method, *e.g.* the electrodes being equal, if R_a is the resistance due to the electrode A , R_b that due to the electrode B , and R_c that due to the electrode C ; and if R_{ab} , R_{ac} and R_{bc} are the resistances observed between A and B , A and C , and B and C respectively, we have

$$R_a + R_b = R_{ab},$$

$$R_a + R_c = R_{ac},$$

$$\therefore 2R_a + R_b + R_c = R_{ab} + R_{ac},$$

whence

$$R_a = \frac{R_{ab} + R_{ac} - (R_b + R_c)}{2}.$$

but

$$R_b + R_c = R_{bc}.$$

$$\therefore R_a = \frac{R_{ab} + R_{ac} - R_{bc}}{2}.$$

Similarly R_b and R_c may be determined and R_d will equal $R_{ad} = R_a$.

Obviously R_{bd} and R_{cd} can then be calculated. As will be seen no very good agreement is attained.

Table IV. *Resistances, observed and calculated, on 6" square.*

Exp.	R_{ab}	R_{ac}	R_{bc}	R_{ad}	R_{bd}		R_{cd}	
					Obs.	Calc.	Obs.	Calc.
1	2100	2300	2900	1300	2300	1900	2400	2100
2	2740	3080	4000	1520	2970	2440	3180	2810
3	2380	2630	3500	1400	2670	2270	2800	2520
4	3400	3570	5550	1620	3570	3600	4000	3770
5	2800	3000	4370	1450	2970	2820	3250	3020
6	2490	2900	3850	1420	2720	2370	2970	2780
7	2470	2770	3750	1390	2670	2370	2850	2670
8	3020	3900	4950	1590	3490	2640	3650	3520
9	4160	4250	8000	1490	4400	5240	5500	5330

This method of calculation gave results more in accordance with the observations when seven electrodes were set out in the angular points of a regular hexagon with the seventh in the middle, in such a manner that the distance between any adjacent pair was the same — 18" corresponding to Gardner's limit; the value of an electrode being determined as the mean of the results obtained from two or three triangles. As these results are very lengthy and of no importance in themselves they are omitted. The calculated and observed results agreed to about 100 ohms in 3000. It will be seen later that in an infinite isotropic medium the calculation should give the result desired, the variations are therefore most probably due to local anisotropic conditions.

Resistance at greater Distances. The question of what happens at distances much exceeding 18" was investigated by the author, once as a continuation of the 7" depth observations of Table II and once independently of these. The results, given in Table V, show a slow increase of resistance beyond 18" up to quite long distances. We are therefore driven to conclude that had Gardner actually measured the resistance between two of the smaller electrodes at 15' instead of merely calculating it he would not have concluded that the intervening $13\frac{1}{2}'$ of soil accounted for only 2 per cent. of the resistance at 15', the rest being due to the 9" of soil about the electrodes.

It will be observed that in Exp. 2 there is no minimum at 18". There is here no real discrepancy, the occurrence of a minimum being a possible

and not a necessary phenomenon, as will be shown from theoretical considerations later on in this paper.

Table V. *Resistances at greater Distances.*

Distance	Exp. 1	Exp. 2	Distance	Exp. 1	Exp. 2
4	5200	2400	3'	—	3200
6	4000	2300	4'	4500	3700
9"	4400	2600	6'	5200	4400
12"	4600	3050	8'	—	2250*
18"	3800	3000	9	5400	—
2'	—	3700	12'	—	4300
27"	4000	—	16'	—	5400

* I am unable fully to account for this lower figure; several subsequent tests about the same place gave results in the neighbourhood of 3600.

To summarise the results to this point, we find that the pot experiment appears to favour Briggs' theory, the fact that agreement between calculated and observed resistances, even when the calculation was made from a single triangle, was better in the hexagon than in the square speaks more in favour of Gardner's limit being correct. On the other hand neither theory explains the increase of resistance at distances exceeding 18", the minima observed in Table II, nor why these minima should appear at greater distances as the depth increases.

THE PATH OF THE CURRENT.

Experimental. It seemed probable that a more satisfactory result might be attained by casting overboard the idea, really no more than a convenient fiction, of the resistance being due to the soil in the immediate neighbourhood of the electrodes and considering the whole mass of soil about them.

An experiment was devised the object of which was to determine at what depth below the line joining the electrodes a conducting layer would appreciably affect the resistance between them. For this purpose a beaker of about 600 c.c. capacity (Fig. 2) was taken and four scales of inches were gummed up the sides of it. Inside this lay a clean perforated metal plate of nearly the diameter of the beaker so that the curved-in sides of the vessel would just keep it off the bottom. Through the centre of this plate was passed a glass tube carrying a copper conductor which was soldered to the underside of the plate. At the top, the tube made connection with a funnel by means of about an inch of rubber tube, but the copper wire passed out at the side and the opening was rendered watertight with sealing wax. The beaker was then filled up to the 2" mark with pure dry sand and five subsidiary electrodes of

copper wire were buried therein at the $1\frac{1}{2}$ " level. The rest of the beaker was filled with the same sand very slightly damped so as to have a resistance of about 11,000 ohms between the main electrodes, consisting of solder spheres on the ends of thick copper wires, placed $2\frac{1}{2}$ " apart in the sand at the $3\frac{1}{2}$ " level. The subsidiary electrodes were connected to a ring conductor surrounding the apparatus and this was joined through a galvanometer to a single dry cell the other terminal of which made connection with the wire from the metal plate at the bottom. The main electrodes were connected through the bridge to the secondary of the induction coil. The sensibility of the galvanometer was such that when

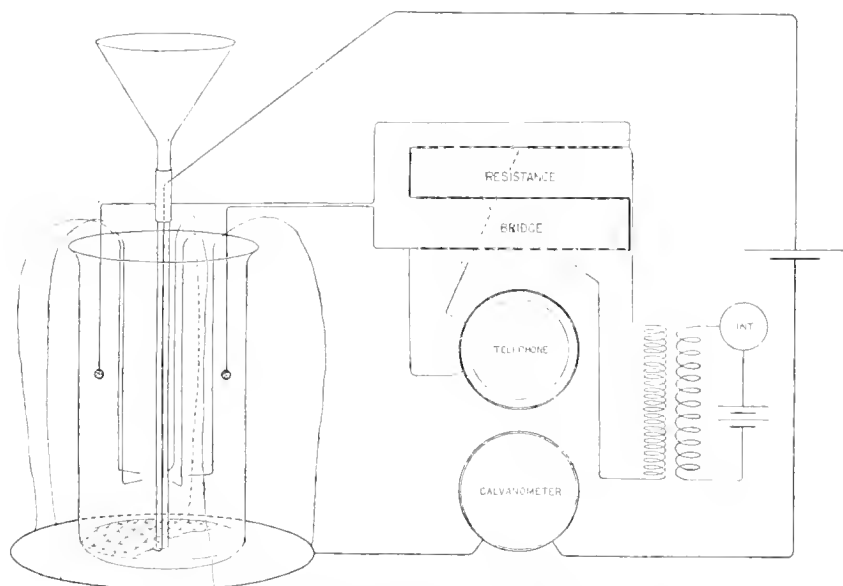


Fig. 2. Apparatus and connections in Beaker Experiment.

one subsidiary electrode was placed in conducting connection with the metal plate the needle moved only a fraction of the distance across the scale, so that all five electrodes would have to be in use to give anything like a complete cross swing.

Hence on pouring a solution of an electrolyte (very dilute hydrochloric acid) down the funnel, it was possible to see if the surface of the solution rising by capillary attraction was sensibly plane or whether it was depressed or humped up at the centre; the condition for a plane surface being that a sudden complete cross swing of the galvanometer needle should be observed at the moment the water line visible on the

outside reached the $1\frac{1}{2}$ " mark. In the experiment this was observed quite sharply and it was therefore assumed that the liquid surface in the capillaries was sensibly plane. The damping of the sand above had not been carried so far as to interfere with packing and it was therefore presumed that this condition was equally satisfied above. The resistance unplugged in the standard arm of the bridge was 10,000 ohms, so that the effect awaited as the solution gradually rose was a sudden cessation of sound in the receiver, followed immediately by a sudden increase. This also gave a very sharp indication when the water line read on the four scales round the beaker was something between $\frac{1}{2}$ " and $\frac{1}{4}$ " below the line of the main electrodes, the readings were $3\frac{1}{8}$ ", $3\frac{1}{4}$ ", 3 ", $3\frac{1}{4}$ ". Thus when the conducting surface reached a level of somewhat more than $\frac{1}{4}$ " below that of the two electrodes the resistance between them fell by about 10 per cent. From this result it appears probable that the current density which is concentrated in the line joining the two electrodes becomes rapidly less on moving away from this part of the field, though probably not as quickly as the result appears at first sight to suggest, as the conductivity is only increased over a comparatively small part of the region surrounding the line of the electrodes.

The following experiment, originally performed as a means of confirming the above result, is described not so much for any value it has in itself, as on account of the justification it seems to supply for the assumptions made in the short mathematical investigation which follows. A tray was taken in which moist sand was laid out in a lamina $\frac{3}{4}$ " thick. Electrodes were placed $6\frac{1}{2}$ " apart in this and the width of the conducting layer was gradually cut down by the insertion of wooden partitions on each side of the line of the electrodes at varying distances. The increase in resistance with decrease in width of the conducting layer was quite in accordance with expectations, becoming much greater as the electrodes were approached, while remaining practically unchanged at considerable distances. The results are given later in Table VI.

Theoretical. It is shown by Mascart and Joubert (a) that the resistance between two electrodes immersed in an unlimited isotropic conducting medium depends only on the medium and the form and dimensions of the electrodes, and is doubled if the medium is limited by an infinite plane passing through the electrodes, *i.e.* if the medium extends on one side only of this plane. On account of the complicated nature of the considerations involved they omit any consideration of the problem when the bounding plane passes to one side of the line of the electrodes. It will suffice for our purpose however if we can determine in some

simple manner the volume of soil through which the practically important portion of the current flows.

The method of attacking the problem adopted by Mascart and Jonbert, viz. by electrical flux and tubes of flow leads to complications when applied to cases other than the two selected. Since we are using an alternating current, we may probably treat the matter from the electrostatic point of view at any one moment, instead of considering it generally from electrodynamic considerations, without any serious error.

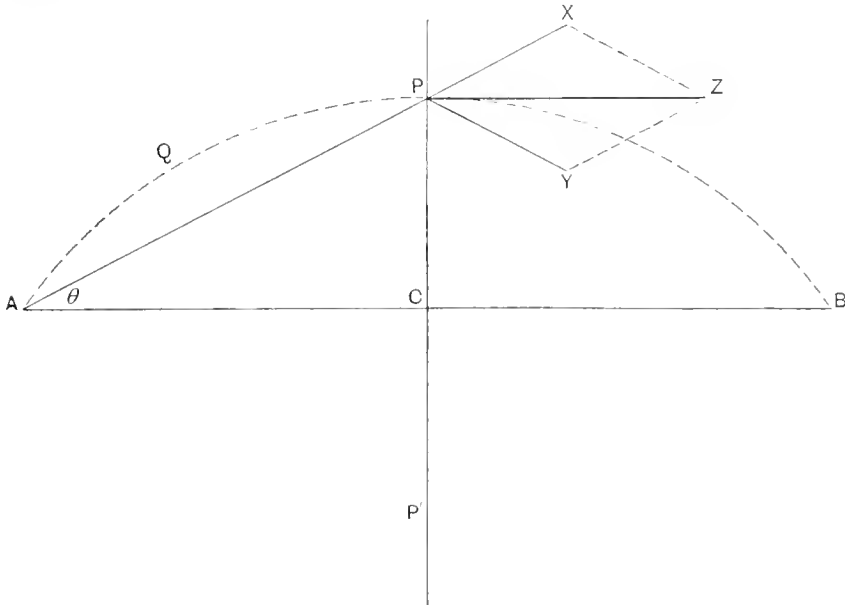


Fig. 3.

Let A and B (Fig. 3) be two electrodes, supposed small, in an isotropic conducting medium. We may assume that at any moment an ion at any point P in the median plane PCP' is subject to two forces, one acting from A , represented in magnitude and direction by PX , and one towards B , represented in magnitude and direction by PY . These will be equal at any point in PCP' and will have a resultant PZ which may be taken to represent the force tending to move the ion at the moment which will be proportional to the current, since assuming Ohm's law, the mean velocity of the ions resolved in the direction in which the force acts varies as the force.

Thus the current density at any point on the median plane PCP' will be proportional to PZ at that point.

Now if the angle $BAP = \theta$ we have in three dimensions

$$PX = PY \propto \frac{1}{(AP)^2} = \frac{1}{(AC \sec \theta)^2} \propto \cos^2 \theta,$$

if AC remains constant.

$$\text{Hence} \quad PZ = (PX + PY) \cos \theta \propto \cos^3 \theta.$$

Precisely the same result is obtained from a consideration of the equipotential surfaces. Since the electrification of the electrodes at any moment will be equal and opposite the median plane is the equipotential surface $V = 0$.

On either side of this will be equipotential surfaces $V = +dV$ and $V = -dV$ and the current across the median plane therefore will be inversely proportional to the distance x along a line of force between these two surfaces¹, since this is a measure of the resistance assuming the medium isotropic. It makes no difference in this consideration whether we take the momentary state or put V in virtual volts and the current in virtual amperes. We have then that the current across the median plane at the point P , is equal to $K \frac{2dV}{x}$.

Now considering spheres round the electrodes $V = \frac{Q}{kr}$ where r is the radius of the sphere, whence

$$dr = -\frac{k}{Q} r^2 dV,$$

and since x is inclined at an angle $= \theta$ to the direction of r

$$\begin{aligned} x &= dr \sec \theta \\ &= -\frac{k}{Q} r^2 \sec \theta dV \\ &= -\frac{k \cdot AC}{Q} \sec^3 \theta dV. \end{aligned}$$

Hence when the potential difference at the ends of x is constant $= 2dV$ we have

$$x \propto \sec^3 \theta,$$

whence current across the median plane at $P \propto \cos^3 \theta$ as before.

Thus the fraction of the total current passing *outside* any *tore*²

¹ This may be considered a straight line.

² The lines of force are known to be circular arcs *vide* Mascart and Joubert, *Electricity and Magnetism*, 1, p. 205.

formed by the revolution of a line of force $AQPB$ about the axis AB will vary as:

$$\int_{\theta}^{\pi} \tan \theta \cos^3 \theta d\theta$$

(since the circumference of the tore in the median plane is $2\pi CP \propto \tan \theta$)

$$= \int_{\theta}^{\pi} \sin \theta \cos^2 \theta d\theta$$

$$= - \int_{\theta}^{\pi} \cos^2 \theta d(\cos \theta) = \frac{1}{3} \cos^3 \theta.$$

Thus the fraction of the total current passing *outside* any tore $\propto \cos^3 \theta$ and the fraction passing *inside* any tore $\propto (1 - \cos^3 \theta)$.

It follows from this that 35.7 per cent. of the current passes *outside* the sphere whose poles are the electrodes.

Proceeding in a similar way we have for a lamina

$$PX = PY \propto \frac{1}{AP} = \frac{1}{AC \sec \theta} \propto \cos \theta$$

if AC is constant.

Therefore the fraction of the total current passing *outside* any portion of the lamina enclosed by two lines of force of equal length on opposite sides of AB varies as

$$\int_{\theta}^{\pi} \cos^2 \theta d\theta$$

$$\frac{1}{2} \int_{\theta}^{\pi} (1 + \cos 2\theta) d\theta = \left[\frac{1}{2} \theta + \frac{1}{4} \sin 2\theta \right]_{\theta}^{\pi} = \frac{\pi}{2} - \frac{\theta}{2} - \frac{\sin 2\theta}{4}.$$

Table VI. *Results of Tray Experiment, Observed and Calculated.*

Extent of sand on each side of line of electrodes	Resistance		
	Mean of three actually observed	Corrected for temperature and evaporation	Calculated from the above formula
∞	—	—	18,400
7 $\frac{1}{4}$ "	18,500	18,600	18,600
5 $\frac{1}{8}$ "	19,100	19,500	19,800
4 $\frac{1}{5}$ "	20,100	20,900	20,700
3 $\frac{1}{5}$ "	20,800	22,000	22,200
2 "	23,400	25,000	24,400
1 $\frac{1}{5}$ "	27,100	29,700	36,800

As will be seen from Table VI this agrees very well with the results obtained in the experiments with the tray of moist sand described above.

220 *The Electrical Method of Soil Moisture Determination*

The observation at $1\frac{3}{8}$ " is doubtless affected by the nearer approach to a three dimensional condition and the fact that the electrodes were not small in comparison with the width of the field thus restricted. The correction for evaporation was made by interpolation from the original and a final observation at $7\frac{1}{4}$ ". We see then that no grave error is likely to have been introduced by the assumptions made at the beginning of this section.

This being so we are probably not far wrong in interpreting our soil phenomena in terms of the calculation made for the three dimensional case, more especially as it explains all the phenomena observed. Each electrode may obviously still be considered to possess a definite resistance value of its own at any stated distance, and calculations based on these values will still yield approximately correct results but slight differences will doubtless appear according to the direction in which the second electrode lies. Since with increasing distance apart local differences in specific resistance will tend to cancel out in their effects it is clear why a better agreement was obtained in the hexagon than in the square. The result of the pot experiment is explainable on either theory, but it is noteworthy that the theory agrees with the halving of the resistance at 4" depth. Calculation actually shows an external residue of current of about 8 per cent, but since the surface is plane it follows that a considerable portion of this would, in the circumstances of the experiment, be included. The steady but slow increase of resistance as the electrodes are moved further apart beyond 18" is explained by the cutting off by the air layer of regions of increasing current density. Combining this with the fact of the existence of a moisture gradient in the soil we obtain an explanation of the minima observed in the plot trials (Table II) since, starting with the electrodes in close proximity and gradually moving them apart the first effect is to cause a fall in resistance by causing the current to dip down into lower moister layers. After a time however, with increasing distance, the non-conducting air layer above begins to cut into regions of greater and greater current density till the resistance introduced by this more than compensates for the other effect. In the second series of experiments in Table V there is no minimum. These were made at the end of the long drought of 1921 and it appears probable that the second effect overbalanced the first from the beginning, owing to the moisture gradient in the soil being less steep. An explanation is also afforded of the increase of the distance apart at minimum resistance with increasing depth since it will in this case be necessary to move the electrodes further apart before the air

layer begins to affect the conduction between them to a similar extent. The result obtained in the beaker experiment is easily understood since the surface of the conducting layer being plane, only small segments of tores carrying an appreciable fraction of the current pass through this layer, and the segment is less the greater the current density in the tores. An attempt to solve the matter completely by mathematics led in all cases to expressions integrable only over restricted ranges or in very slowly converging series, putting this method of procedure out of court for practical purposes. A series of rough approximations led to results not out of keeping with those obtained.

CONCLUSIONS FROM ABOVE EXPERIMENTS.

These results are of practical importance as they show what may and what may not be expected from this method of moisture determination. It is clear in the first place that what is obtained is the mean resistance of a volume of soil which may for practical purposes be considered as of rather greater extent than a sphere whose poles are the electrodes, something the shape of an apple with the electrodes at the calyx and stalk, since the fraction of current passing outside a selected tore falls off very sharply beyond $\theta = 45^\circ$, and soon becomes negligible. The moisture content will also refer to this volume of soil and local and depth variations will be obliterated. If therefore we desire to measure the moisture content at any depth we must place the electrodes at such a distance apart that the moisture gradient can be considered uniform in the volume of soil concerned when the deficiency above the level of the electrodes will be counterbalanced by the excess below this level. Moreover if the distance is more than a few inches it will be necessary to take account of the tendency of the current to dip down into the moister layers, or to continue the simile above, of the downward bend in the core of the apple. Secondly the electrodes should really be of such a size that they may be considered small in comparison with the distance apart as otherwise the calculations do not strictly hold and the new conditions ought to be investigated afresh. The necessity of obtaining good contact with the soil puts a practical limit to the size of the electrode and it was found in consequence that 3" apart is about the lower limit for the electrode distance. The distance apart must moreover be chosen with a view to confining the current or such part of it as matters to the portion of soil concerned, *e.g.* a distance of 15'-20', while probably giving illuminating information on forest land would usually be quite useless in ordinary arable farming. Finally it seems possible that considerable

222 *The Electrical Method of Soil Moisture Determination*

use might be made of the method in arid sandy regions for determining the approximate depth at which the water table lies, *e.g.* supposing the depth of this were 50'—starting with electrodes 6" deep say and a short distance apart on increasing the distance the resistance would gradually increase to a point but on reaching a distance apart of about 200' the beaker experiment would lead us to expect a fairly sharp fall due to the water at 50' depth. A few observations would suffice to determine at what fraction of the distance apart when the resistance falls the water table would be found. It is perhaps worthy of note in this connection that the resistance of earth returns in telegraphy is very low; though of course, as large plates are usually buried for this purpose, we should not expect anything like the resistances encountered in the soil experiments.

RESISTANCE-MOISTURE CURVES.

Experiments were made in the laboratory to determine the character of the resistance-moisture curve. Artificial mixtures, natural sands and soils were employed and results obtained in accordance with those of Whitney (*l.c.*), except that at low moisture contents definite discontinuities were observed.

The method of carrying out the experiments was alike in all cases. 500 grams of the soil or other substance used was placed in a wooden box whose internal dimensions were $2\frac{3}{4}" \times 6" \times 2\frac{1}{2}"$ and the electrodes, small carbon cones, were placed at each end. An extra amount of the same sample was used as a reserve from which samples removed for moisture determinations could be replaced, maintaining the original weight of dry soil. The hygroscopic moisture was found not to affect the resistance within the capacity of the instruments used; that is to say the air dry substance had in all cases a resistance under the conditions of experiment exceeding one megohm. As wetting a sample in such a way as to render it only slightly moist is difficult the following plan was adopted: the sample was wetted slightly with distilled water and then thoroughly mixed, after which it was warmed to about 30° C. and allowed to cool again once or twice. This seemed to distribute the moisture evenly through the mass and was preferred to the alternative of gradually drying out a saturated mass as its character is changed by this treatment. The damp sample was then placed in the box and packed down to a constant level¹—a thermometer placed in the middle of the mass enabled the temperature to be read and the resistance was taken

¹ Great difficulty was found in this in the case of the boulder clay: the observations were less satisfactory on this account.

when this had fallen to some point but slightly above room temperature, the same temperature being employed throughout any one series of determinations. A sample was taken out from the middle of the mass after each resistance reading and the moisture in it determined by drying at 110° C. About 15 to 20 determinations were made in this way for each sample. It was not, however, found possible to carry the moisture content up to saturation since near this point the water tended to ooze out from the bottom of the box. The results obtained with one soil are given in Table VII. If plotted out they yield hyperbolas, but if, instead of

Table VII. *Results of Resistance Moisture Determinations on a typical soil (Greensand).*

Hygroscopic moisture 1.4 %			
Moisture % dry weight	Resistance in ohms	Moisture % dry weight	Resistance in ohms
2.3	1,100,000	7.1	16,800
2.9	390,000	7.6	11,700
3.4	257,000	8.9	8,920
3.6	208,000	9.6	6,200
4.4	130,000	10.7	5,050
4.9	114,000*	11.6	3,950
5.1	60,000	12.5	3,490
5.3	91,500*	13.0	3,270
5.7	39,000	14.2	2,500
5.8	70,900*	18.6	1,870
6.6	19,200		

plotting directly, we plot the logarithm of the moisture expressed in percentage dry weight, less the hygroscopic moisture, against the logarithm of the resistance observed we obtain the curves shown in Figs. 4, 5 and 6: whence it is clear that at low moisture contents the curve is discontinuous at any rate at one point, the existence of the first point of discontinuity is disputable owing to the small number of observations. It will be observed that the type of curve is different for the artificial mixtures to what it is for the natural soils and sands taken. In the former case the resistance falls sharply at first, then less so and finally sharply again, in the latter the reverse holds—slowly at first, then more quickly, and slowly at the end.

It would appear that there ought to be no sharp angle between the different regions as shown in the curves. It was felt however that as the accuracy of the measurements was not sufficiently great to justify the use of them as a basis for the discussion of this matter greater clearness would be attained by drawing the lines as shown.

The facts seem explainable as follows:—In the case of pure sand and artificial mixtures we may assume that there is no measurable amount

of colloid present, while in the natural calcareous sand (coarse) a mechanical analysis showed 0.45 per cent. clay and the other sands and soils used contained considerably more than this. Now in the case where we have no colloid present we should expect little or no reduction of resistance until the 50μ film thickness of Quincke(7) is passed since Terzaghi(8) has shown that there is no brownian movement and therefore presumably but little if any ionic movement in this layer. Owing to evaporation it was found impossible to obtain any results in this region.

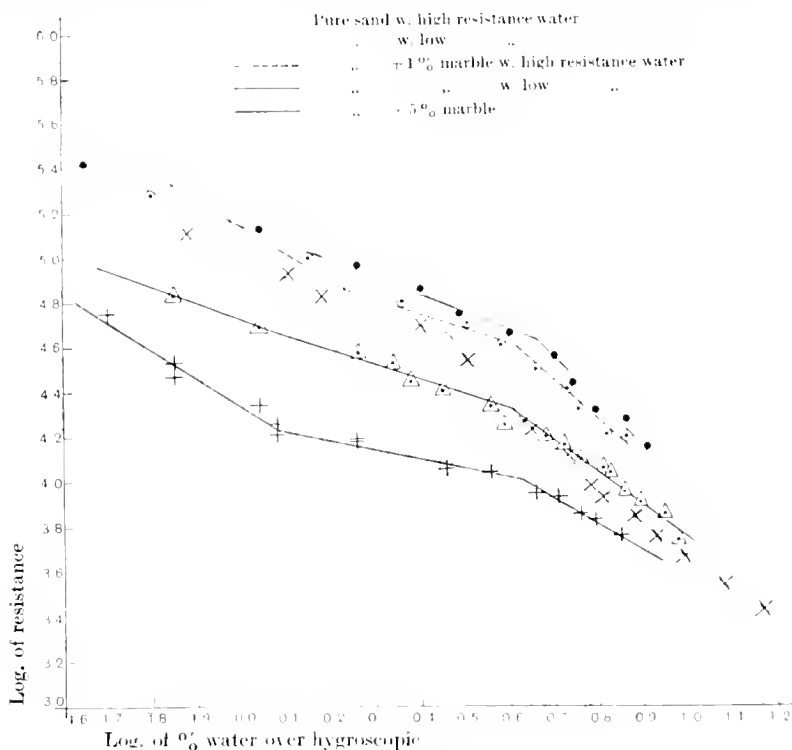


Fig. 4.

Beyond this the addition of water might be expected to cause a more rapid fall than in the third stage where it will be sensibly inversely proportional to the moisture content up to the point at which surface tension begins to overcome the force of adhesion¹. When surface tension

¹ For in this case the surface of electrode wetted will be directly proportional to the thickness of the moisture layer, thus if R is the radius of a particle, supposed spherical, and ΔR the thickness of the water film upon it and if n is the number of contacts between

begins to take effect however the tendency is to concentrate the extra moisture added in the interstices where one particle touches another or the electrode surface and therefore we may expect that the resistance will vary inversely as some function of the moisture content. Experimentally it was found to vary approximately as the inverse square of the moisture as had been found previously by Whitney.

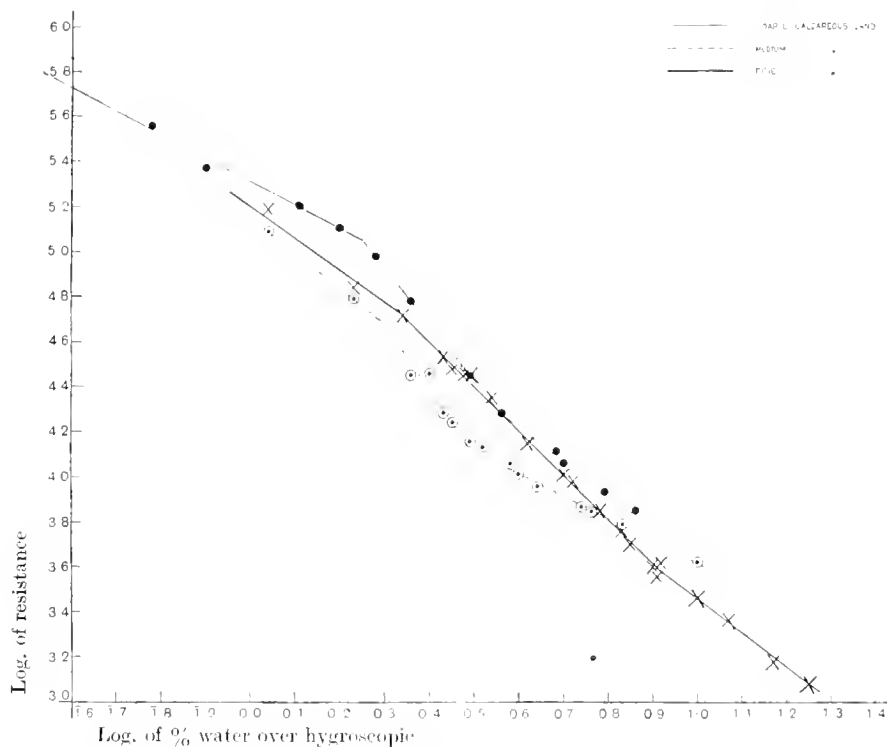


Fig. 5.

A tentative explanation of the second series of cases where the particles are covered by a colloid substance was originally elaborated on the theory that the conductivity of a substance in the gel stage would be less than that of the same substance under similar conditions as a sol. Recent work by Miss Laing and Prof. McBain⁽⁹⁾ however shows that

the several particles and the electrode the area wetted $\propto n[(R + \Delta R)^2 - R^2] = 2nR \cdot \Delta R$ neglecting higher powers of ΔR . If m is the moisture content $nR \cdot \Delta R \propto nRm$. But for any electrode obviously $n \propto \frac{1}{R}$. \therefore Resistance $= \frac{1}{\text{conductivity} \propto \text{area wetted} \propto \frac{1}{m}}$. The experimental results agree with this in the region dealt with.

in the case of sodium oleate the gel stage has under similar conditions of concentration and temperature the same conductivity as the sol. The similarity of nature between this and the silica gel, with which we are most probably dealing, suggests that a like phenomenon would be observed here. Fortunately Laing and McBain's work itself suggests an alternative explanation. They found the conductivity of the soap-curd was much lower than that of the transparent gel or sol and distinguish

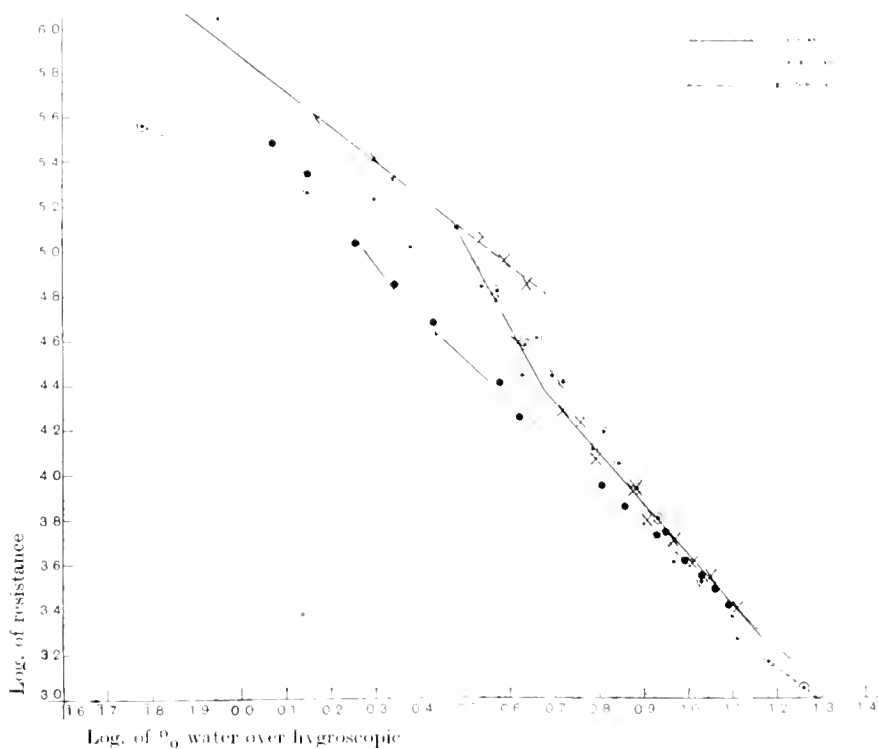


Fig. 6.

sharply between coagulation and gelation. Thus in the first stage we may be merely increasing the degree of hydration of the fibres of the silica coagulum, in the second stage where the curve descends more steeply we have a passage from a coagulum to a gel in which the resistance falls not only on account of the water added but also because the specific resistance of the gel is less than that of the coagulum, finally when the coagulum is all converted into gel we have the third stage due to progressive dilution of the gel or sol, it being immaterial which is present.

The principal objection to this is the fact that no stage corresponding to the curd stage in soaps has been observed in silica or other similar gels like gelatine; but if the curd fibres in soap had been transparent instead of white it is by no means certain that they would have been observed, and in this case since silica crystals are transparent while soap crystals, of which the curd is thought to consist, are white, it seems not impossible that silica curls may exist in the gel under certain conditions and yet have remained unnoticed up to the present; only further experiment can decide the matter. Laing and McBain found the specific conductivity of the curd to *increase* with concentration, but as the highest concentration employed was $\cdot 6 N$ no deduction germane to the present case can be made from this.

It is worth mentioning that the first kink in the curve seems to be related in some way with the "unfree" water of Bouyoucos⁽¹⁰⁾. The mean of his values for various sands is 1.6 per cent. which is in good agreement with the moisture at the first kink in the sands dealt with here, which varies from 1 to 2 per cent. In the soils we have greensand 4.4 per cent., glacial loam 3.2 per cent. and boulder clay 8.4 per cent. at the first kink. Bouyoucos' averages for unfree water in sandy loams and clays come out 4.4 per cent. and 12.2 per cent.

As regards the effect of the resistance of the distilled water used with artificial mixtures this is quite what one would expect. In natural sands and soils, salts are present in sufficient quantity completely to mask any effect due to this cause.

It appears then, that for any pair of electrodes it should be possible to construct an empirical curve from which, given the resistance between them in any definite place the moisture content can at once be deduced. The degree of accuracy attainable in practice would probably not be as great as the lie of the observations about the curves would lead one to expect as, apart from the error in sampling a large volume of soil for standardisation purposes, the hysteresis effects on wetting and drying the colloids would undoubtedly have some effect. Moreover, the curve obtained for the greensand seems to indicate another possible source of error which might prove very serious. The portion drawn with a dotted line¹ was obtained quite by accident, after the last observation on this, an abnormal fall in resistance was noted—the author therefore went backwards and obtained the full line curve. On adding water afterwards this curve was consistently followed and it was found impossible to proceed again along the dotted portion. Thus there seems to be a possibility

¹ Corresponding observations marked with an asterisk in Table VII.

228 *The Electrical Method of Soil Moisture Determination*

under exceptional circumstances of getting a metastable condition of some kind; possibly due, if the theory adumbrated is true, to something of the nature of a supersaturation of the coagulum—or, more probably, a time factor comes in, Laing and McBain having found that the conductivity of a newly formed curd falls off for a long time after its formation, thus we might expect a similarly retarded recovery if water is added too rapidly. The probability of this is increased by the fact that these workers found that the best way to obtain the gel was to warm the curd *very slowly*. This error would in this case be unlikely to appear in soil work.

The agreement of the last portion of the curve as to slope in all cases seems to show that in general at moisture contents exceeding 10 per cent. or thereabouts the American formula holds good approximately and we may therefore use it in the form given by Gardner, viz.:

$$W_1 = \sqrt{\frac{W^2 R}{R_1}}$$

where W = per cent. moisture at time of standardisation,

R = the resistance corresponding to W ,

R_1 = the resistance observed,

and W_1 = the per cent. moisture corresponding to the resistance R_1 , provided always that we do not lose sight of the sources of error noted above and of the limitations of the method dealt with earlier in this paper.

There remains the question of the effect of movement of soil salts in the soil which, if considerable, may either vitiate the method altogether, or render it too cumbersome for use by necessitating a too frequent standardisation of the electrode. Experiments are proceeding on this matter, but from the extreme slowness of the method adopted for this purpose, the full results are not likely to be available for some time. It may be stated however that the material so far to hand seems to indicate that where a soil is protected from rain these salt movements do not affect the moisture determination by as much as one half per cent.

I take this opportunity of thanking all those who by their unfailing interest, help and encouragement have enabled me to bring this investigation to its present position. Among these I owe a special debt of gratitude to Mr J. W. Capstick, O.B.E., M.A., D.Sc. for continuous advice and sympathetic criticism throughout the whole time that the experiments were in progress.

SUMMARY.

In this paper an examination is made of the processes operative and the limits of accuracy of the electrical method of determining soil moisture.

The resistance over a small plot is found to vary under similar conditions and it is concluded that these differences are most probably due to actual differences in moisture or other factors.

The effect of the distance apart of the electrodes is investigated and a probability of a minimum resistance between two electrodes being observed under certain conditions is indicated.

The use of electrode values in computing soil resistances is discussed and criticised.

The path of the current in the soil is investigated mathematically and it is shown that the results obtained accord well with the observed facts.

It is concluded that the method gives the mean water content of a volume of soil somewhat greater than a sphere whose poles are the electrodes. The practical limits of the method are indicated.

Certain resistance-moisture curves obtained in the laboratory are discussed and it is concluded that while at water contents above 10 per cent. the relation found by the American investigators holds good—viz. that the resistance varies inversely as the square of the moisture content; at lower water contents than this one and possibly two discontinuities appear in the curve.

These discontinuities are reversed in the case of artificial mixtures not containing colloids.

A tentative explanation of these phenomena is given.

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230 *The Electrical Method of Soil Moisture Determination*

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THE CHEMISTRY OF THE STRENGTH OF WHEAT FLOUR.

By HERBERT ERNEST WOODMAN, PH.D., D.Sc.

*(From the Animal Nutrition Institute, School of Agriculture,
Cambridge University.)*

It is well known that different flours vary enormously in respect of the size and shape of loaf they yield on baking. The factor which determines the quality of flour in this connection has been termed "strength" and the latter has been defined as "the capacity of flour for making large well-piled loaves" (1).

Many views have been held from time to time regarding the explanation of flour strength from the chemical standpoint. The earliest view was that strength was determined by the gluten content of the flour, which by virtue of its tenacity was able to retain in the bread the carbon dioxide produced as a result of the activity of the yeast. Many cases, however, were investigated where flours possessing a high gluten content were not so strong as a flour with a low content of gluten. Furthermore, no accepted regularity has been found to exist between the strength of flours and the water-holding or gas retaining capacity of their glutens.

Attention was next directed to the consideration of the individual proteins in the gluten of flour, namely, gliadine and glutenine. It was found that measurements of the absolute amounts of gliadine showed no correlation with strength. Neither was it possible to show any consistent relationship between strength and the ratio of gliadine to glutenine in the gluten. It was suggested by Hall(2) that gliadine might not be a definite substance and that the gliadine contained in very strong flours might be different from that in weak flours. Wood(3), however, prepared and examined samples of gliadine from strong and weak flours and concluded, on the grounds of their content of amide nitrogen, that the proteins from both sources were identical. Indirect determinations of the amide nitrogen of the glutenines of weak and strong flours led to the conclusion that the glutenine protein in different flours was one and

the same substance. It was therefore not possible, on the available evidence, to explain the difference in the loaf-making qualities of different flours by reference to their protein content.

A valuable contribution to the study of the subject was made, when Wood(3) resolved the conception of flour strength into two factors:

1. The factor of strength which determines the *shape* of the loaf.
2. " " " " " *size* " "

The results obtained by this investigator justified the conclusion that the capacity of a flour for giving off gas when incubated with yeast and water is the factor which in the first instance determines the *size* of the loaf. The latter depends not so much on the amount of sugar present in the flour as such, but on the diastatic capacity of the flour, which gives rise to continued sugar formation and consequently continued gas evolution in the dough. The same worker also showed that the properties of gluten in regard to coherence and elasticity were subject to considerable modification by the concentration of acid, alkali or salt in the solution with which it was in contact, and he suggested that these properties have an important bearing on the *shape* of the loaf. A knowledge of the acidity and soluble salt content of a flour should therefore afford a clue to that factor of strength which decides whether the flour will make a good-shaped loaf.

The information obtained in the investigation referred to above has been applied with success in several phases of the milling industry. The view has long been held, however, by Professor Wood himself, that in view of the improvement of the methods employed in protein research, the question of the identity or non-identity of the corresponding gluten proteins in weak and strong flours should be re-investigated. It is now recognised that two proteins may be quantitatively identical with regard to their amino-acid content and yet be two distinct proteins, by virtue of differences in the order of linkage of the amino-acids within the protein molecules. Such a case is furnished by the caseinogens of cow's and sheep's milk. Though it is possible to show that these proteins are distinct substances(4), yet by the ordinary chemical methods of analysis they are indistinguishable.

It is clear, then, that any chemical method which is to be employed to decide on the identity or non-identity of related proteins must be such as to take into account the possibility of differences connected with the order in which the constituents of the proteins are linked up within the molecules. Such a method is the Racemisation Method, which has been used recently in the investigation of the corresponding proteins of

blood serum, colostrum and milk(5). The method depends on the behaviour of proteins in dilute alkaline solution. When such solutions are kept at 37° C., they suffer a progressive diminution in the value of their optical rotatory power as a result of a keto-enol tautomerism of the $=CH-CO-$ groups in the protein complex. If the specific rotations of the solution be plotted against the time in hours during which the reaction has been allowed to proceed, then the readings fall on a perfectly smooth curve. It is found that the rotation sinks rapidly at first, then more slowly and subsequently after about 250 hours attains a practically constant value. The process thus results in the partial racemisation of the protein, and the graphs thus obtained are referred to as racemisation curves.

Since individual proteins display specific behaviour quantitatively when racemised with dilute soda, it follows that the method may be used to test the identity or non-identity of related proteins. Thus, if two proteins are to be pronounced identical, then if racemised under the same conditions, their solutions must show the same initial rotation, the same final rotation and the same rate of diminution of rotation. They must also continue to display identical optical behaviour if the concentration of the alkali or protein in the solution is varied. On the other hand, if two proteins are not identical, this will be revealed by their possessing distinct sets of racemisation curves.

In the investigation to be outlined in the present communication, samples of gliadine and glutenine have been isolated from typical strong and weak flours and have been investigated comparatively by means of the racemisation method. The results obtained are very suggestive in relation to their bearing on the existing ideas of flour strength, since it has been shown that whereas the gliadines from weak and strong flours are identical proteins, yet the glutenines prepared from the same sources appear to be two distinct chemical individuals.

PREPARATION OF PROTEINS.

1. *Gliadine and glutenine from strong flour.*

The flour used for this purpose was a typically strong flour milled exclusively from Northern Manitoba Wheat. The method used for the isolation of the proteins was in its essentials the same as that described by Osborne(6). The bulk of the starch and soluble constituents was removed by enclosing the flour in a muslin bag and kneading the material in a stream of running water, the process being completed by thoroughly

kneading the gluten under a large volume of distilled water. The resultant characteristically sticky gluten was broken up into small pieces and extracted with alcohol, the alcohol added being such as to give, with the water in the gluten, a solvent containing 70 per cent. of alcohol by volume. After standing for 18 hours with frequent shaking, the supernatant alcoholic solution of gliadine was filtered off and the residue was repeatedly extracted with successive portions of 70 per cent. alcohol until the amount of gliadine going into solution was inappreciable.

The combined filtrates were then concentrated *in vacuo* at 50 °C., care being taken to keep the gliadine in solution by adding small portions of alcohol from time to time. The concentrated solution was cooled and poured slowly, with constant stirring, into a large volume of ice-cold distilled water containing about 10 grm. salt per litre. A gummy mass separated out which collected on the glass rod. This was repeatedly washed with distilled water, dissolved in 70 per cent. alcohol and the solution filtered until water clear. After concentrating the solution *in vacuo* at 50 °C., the syrupy residue was cooled and poured into absolute alcohol. It was found that complete separation of the gliadine could only be obtained by this method after stirring a little salt into the alcoholic liquid. The addition of ether also enabled the gliadine to separate completely.

The process of dissolving the gliadine preparation in 70 per cent. alcohol, filtering, concentrating *in vacuo* and precipitating by means of absolute alcohol was carried out in all three times, the final precipitation being effected by a mixture of ether and absolute alcohol, this resulting in the gliadine separating in a flocculent condition. The protein was then dried by washing successively with absolute alcohol and anhydrous ether and was finally obtained as a white powder which dissolved completely in 70 per cent. alcohol to give a water clear solution. As a result of the method of preparation, it contained a little salt as impurity.

The gluten residue, after extraction of the gliadine with 70 per cent. alcohol, was allowed to dry at room temperature and then powdered. It was then further extracted with alcohol, the gliadine-free residue being shaken with successive portions of ether to remove fat. After air-drying, the material was shaken with sufficient 0.2 per cent. KOH to effect solution. The extract was filtered, great difficulty being experienced in obtaining a clear filtrate. From the latter the glutenine was precipitated by means of very dilute hydrochloric acid, the amount of acid requisite for flocculent precipitation being determined by a preliminary test on a small bulk of the alkaline solution. The precipitate

obtained in this manner was exhaustively extracted with 70 per cent. alcohol to remove traces of gliadine. It was then redissolved in the minimum amount of 0.2 per cent. KOH, filtered clear and the glutenine reprecipitated in the manner already described. The process of precipitating the protein from alkaline solution was carried out in all four times, and after each precipitation, the glutenine was extracted with 70 per cent. alcohol to ensure complete removal of gliadine. After the final precipitation, the glutenine was well washed with distilled water and was obtained as a white powder by successive washings with absolute alcohol and anhydrous ether. The preparation gave a water clear solution in 0.2 per cent. KOH and an exhaustive extraction of a sample of the material showed that it was entirely free from gliadine. It contained, as a result of the method of isolation, a small amount of potassium chloride as impurity.

2. *Gliadine and glutenine from weak flour.*

The flour used as the starting point for the preparation of these proteins was one which had been milled exclusively from English wheat. The same methods were employed as those outlined in the preparation of the proteins from Manitoba flour.

The gluten of the English flour differed materially from that of Manitoba flour in respect of its physical properties. Whereas the latter was sticky and coherent, the former lacked coherency and resembled putty in its consistency.

Much bigger percentage yields of the proteins were obtained from the Manitoba flour than from the English flour.

METHOD OF INVESTIGATING THE BEHAVIOUR OF THE PROTEINS WITH DILUTE ALKALI.

The protein samples were first finely ground up and then dried *in vacuo* over calcium chloride for several days. The amount of ash-free protein in each sample was determined by means of the Kjeldahl method, the nitrogen content of the gliadine samples being multiplied by the factor $\frac{100}{17.66}$ and that of the glutenine samples by $\frac{100}{17.49}$.

In the case of the gliadine samples, the first series of determinations were carried out in the following manner. Exactly 1 gm. of the dry protein was weighed out into a 50 c.c. flask containing a little distilled water. 25 c.c. of *N* NaOH (or *N*/2 NaOH as the case may be) were then slowly run in from a pipette. After mixing gently, the volume was

made up to the 50 c.c. mark with distilled water and the flask was placed in an incubator kept at 37° C. When the protein was completely in solution, the liquid was filtered quickly into a small flask, which was then stoppered and kept in the incubator. From time to time, the optical rotation of the alkaline solution was determined in a 1d-polarimetric tube, using a Schmidt and Haensch instrument and sodium light. This procedure was continued for about 300 hours, when the value had become practically constant. Graphs were then constructed showing the progress of the racemisation, the ordinates representing specific rotation values and the abscissae the number of hours during which the reaction had been allowed to proceed.

In carrying out the determinations in the above manner, some delay always occurred in effecting complete solution of the gliadine in the alkali. The difficulty was satisfactorily overcome in the following way. The 1 gram. sample of dry protein in the 50 c.c. flask was first dissolved in 10 c.c. of 70 per cent. alcohol. The 25 c.c. of standard soda were then slowly run in, the flask being gently shaken during the process. The volume was then made up to 50 c.c. with distilled water and the flask was placed in the incubator. A clear solution was obtained in a few minutes by this method.

The racemisation data of the glutenine samples were obtained in a somewhat similar manner. 1 gram. samples of the dry protein were weighed into 50 c.c. flasks containing a little distilled water. The 25 c.c. of standard soda were then run in slowly and the flask was gently shaken. A jelly like mass was first obtained, which quickly liquefied when the flask was put in the incubator. The volume was then made up to the 50 c.c. mark with distilled water and the polarimetric readings were taken as described above.

INVESTIGATION OF THE GLIADINE SAMPLES.

1. *Specific rotations in 70 per cent. alcohol.*

2 per cent. solutions of the gliadine preparations in 70 per cent. alcohol were examined by means of the polarimeter with the following results:

Gliadine from Manitoba flour	$[\alpha]_D =$	93.60 .
.. .. English	$[\alpha]_D =$	93.78 .

Osborne(6) gives the value of -92.28° for the specific rotation of gliadine in 80 per cent. alcohol.

It will be noted that the gliadines from the strong and weak flours display no difference in respect of their rotations in 70 per cent. alcoholic solution.

2. *Data obtained in the racemisation of the gliadine samples.*

1. Manitoba flour gliadine 2 % in N/2 NaOH		2. English flour gliadine 2 % in N/2 NaOH	
Time in hours	Specific rotation	Time in hours	Specific rotation
2	-110.5	2	-111.0
5	-106.8	5	-106.3
9	-104.1	9	-104.0
24	-96.8	24½	-96.3
49	-89.0	49	-89.5
74	-84.1	73	-83.9
101	-78.8	101	-79.5
125	-75.1	124	-75.6
169	-71.5	170	-71.3
242	-67.3	240	-67.0

In the series of determinations recorded under 3, 4, 5 and 6, the weighed out samples of protein were first dissolved in a measured volume of 70 per cent. alcohol as described before the addition of the standard alkali.

3. Manitoba flour gliadine 2 % in N/2 NaOH (alcohol present)		4. English flour gliadine 2 % in N/2 NaOH (alcohol present)	
Time in hours	Specific rotation	Time in hours	Specific rotation
1	-109.4	1	-109.0°
5	-98.3	5	-97.8
9	-93.0	9	-93.2
24	-86.2	24	-86.7
48	-80.5	48	-80.6
74	-76.2	72	-76.2
125	-72.6	124	-72.1
170	-69.4	170	-68.9
240	-66.0	242	-65.6

5. Manitoba flour gliadine 2 % in N/4 NaOH (alcohol present)		6. English flour gliadine 2 % in N/4 NaOH (alcohol present)	
Time in hours	Specific rotation	Time in hours	Specific rotation
1	-110.5°	1	-110.8°
5	-103.1	5	-103.1
9	-100.0	9	-99.3
25	-94.7	24	-94.3
48	-90.5	48	-90.5
96	-88.4	97	-88.0
119	-86.8	120	-86.5
168	-84.7	170	-84.0
244	-82.0	242	-81.7

The above determinations were carried out at least in duplicate and the results were confirmed by independent investigation of further samples of the two gliadines prepared from the same flours.

It will readily be seen from the above data that the gliadines from the two types of flour display throughout identical optical behaviour when racemised by dilute alkali at 37°. This fact is brought out more strikingly if the graphs representing the course of racemisation be constructed. For each set of experimental conditions, it will be found that one smooth curve can be drawn to satisfy equally the two sets of readings for the Manitoba and English flour gliadines. Such slight discrepancies as may occur fall within the general error of experiment.

When racemised by means of $N/2$ NaOH, each gliadine solution possesses an initial specific rotation of about -111° (taken from graph¹). The rotations of both solutions diminish fairly rapidly and at an equal rate during the first 24 hours; the rate of diminution in each case then falls off equally and after 240 hours, when the value of the rotation has become practically constant, the two solutions possess an equal specific rotation of about -67° .

The presence of the small amount of alcohol in the second series of determinations exerts a striking effect on the rate of racemisation and affects both gliadines in an equal degree. The effect is best studied by constructing the graphs. The rate of racemisation during the first 24 hours for $N/2$ NaOH is much quicker when alcohol is present than when it is absent. In the presence of the alcohol, the specific rotation during the first 24 hours sinks from -111° to -86° , whereas when no alcohol is present, the corresponding diminution is from -111° to about -97° . After about 50 hours, however, the two curves begin to come together again and almost coincide after about 250 hours. The presence of alcohol causes $N/4$ NaOH to effect a greater reduction in specific rotation during the first day than does $N/2$ NaOH without alcohol. These curves cross after about 35 hours and from this point the rate of diminution with the weaker alkali is relatively slow. The reason for the effect thus produced by the alcohol, which resembles that of a catalyst, requires further investigation. For the purposes of this enquiry, it is sufficient to note that under the three different sets of conditions, the gliadines from the weak and strong flours display the same optical behaviour during racemisation. This fact is taken as evidence of the identity of the two gliadines. A final test on a mixed sample of the two

¹ The graphs are not reproduced here, as they are not necessary in enabling the essential conclusions to be drawn.

gliadines gave readings which fell on the racemisation curves which the two proteins have been shown to possess in common.

3. *Combining weights of the gliadines.*

Foreman(7) has shown that aqueous-alcoholic solutions of certain amino-acids containing about 85 per cent. alcohol can be titrated accurately with alkali in the presence of phenolphthalein. The amino groups under these conditions display no basicity to phenolphthalein and the carboxyl groups can therefore be estimated by titration.

On the basis of this observation, it seemed probable that the basic effect of the free amino groups in the gliadine molecule towards phenolphthalein might be held in abeyance when the protein was dissolved in alcohol, thus permitting of the direct estimation of the free carboxyl groups in the molecule by titration with alkali. Accordingly, 2 per cent. solutions of the gliadines in 80 per cent. alcohol were titrated with $N/10$ NaOH in the presence of phenolphthalein, and the following results were obtained:

Manitoba flour gliadine	1	gram. dry, ash-free protein required	1.95 c.c.
		$N/10$ NaOH. Combining weight =	5128.
English	„	1 gram. dry, ash-free protein required	1.99 c.c.
		$N/10$ NaOH. Combining weight =	5026.

It follows that both gliadines possess the same combining weight, the difference recorded being due to experimental error. This fact affords further confirmation of the identity of the gliadines.

The data recorded above possess an additional interest when considered in conjunction with the minimum molecular weight ascribed to gliadine by Osborne(8) on the assumption that the molecule contains five atoms of sulphur, viz. 15560. It would thus appear probable that the gliadine molecule contains three or a multiple of three free carboxyl groups.

INVESTIGATION OF THE GLUTENINE SAMPLES.

1. Specific rotation in $N/25$ NaOH.

0.5 per cent. solutions of the glutenines in $N/25$ NaOH were examined by means of the polarimeter with the following results:

Glutenine from Manitoba flour	$[\alpha]_D = -99.5^\circ$.
„ „ English	$[\alpha]_D = -78.8^\circ$.

2. Similar differences in the optical properties of the glutenines were observed during the racemisation of the proteins by means of $N/2$ NaOH at 37° C.

240 *The Chemistry of the Strength of Wheat Flour*

Manitoba flour glutenine 2% in $N/2$ NaOH		English flour glutenine 2% in $N/2$ NaOH	
Time in hours	Specific rotation	Time in hours	Specific rotation
1	93.0°	1	-74.0
5	85.0	5	-67.5
9	79.5	9	-62.9
24	-71.0	24	-57.0
48	-60.5	49	-50.0
72	-55.5	72	-45.9
96	-51.2	98	-41.5
143	-47.0	144	-37.0
190	-44.0	192	-35.0
240	-41.5	242	-32.5

3. The following data show the behaviour of the glutenines when racemised with $N/4$ NaOH at 37° C.

Manitoba flour glutenine 2% in $N/4$ NaOH		English flour glutenine 2% in $N/4$ NaOH	
Time in hours	Specific rotation	Time in hours	Specific rotation
2	-94.0°	2	-77.0
9	-85.5	9	-71.6
24	-78.6	25	-65.0
96	-67.8	96	-57.5
144	-63.5	143	-54.0
242	-57.0	240	-48.5

The differences in optical behaviour displayed by the glutenines during racemisation with $N/2$ and $N/4$ NaOH point to the conclusion that the glutenine of strong flour is a different protein from that contained in weak flour. Two objections, however, may be raised against the evidence on which this conclusion is based.

1. In view of the difficulty of effecting complete separation of two proteins from each other, it is possible that the samples of glutenine are still associated with small amounts of gliadine, and that the high rotation values obtained with Manitoba flour glutenine as compared with English flour glutenine may be explained on the grounds that the former glutenine contains more of the high rotating gliadine than does the latter.

2. It has not been demonstrated that the glutenines are extracted from the gluts without change. It is possible that the 0.2 per cent. KOH used in the isolation of the glutenine may cause, even at the room temperature, a slow racemisation of the proteins and thus render uncertain any conclusions which may be drawn as a result of the optical behaviour of the final samples with dilute alkali at 37° C.

The first objection cannot be sustained, however, since a study of the initial rotations of the proteins in alkali shows that the Manitoba flour glutenine would have to contain relatively large amounts of gliadine to account for its optical behaviour on this assumption. Ex-

haustive extractions of the sample with 70 per cent. alcohol failed to reveal the presence of even traces of gliadine.

In view of the second possible objection, the following tests were carried out. A 0.5 per cent. solution of Manitoba flour glutenine in 0.2 per cent. KOH, to which was added a drop of toluene, was allowed to stand at room temperature for about a month and the specific rotation was determined from time to time. The initial specific rotation was -95.0° , and during the period of the trial this value did not suffer any measurable diminution. It is reasonable to assume, therefore, that the optical properties of the glutenines were not affected as a result of their mode of extraction by means of 0.2 per cent. KOH.

Moreover, the rate of diminution of rotation of a 0.5 per cent. solution of Manitoba flour glutenine in $N/25$ NaOH when kept at 37°C . was exceedingly slow, as is evidenced by the following series of determinations:

Time in hours	Specific rotation
2	-99.0°
25	-97.0
98	-91.0
267	-79.0

It will be observed that the specific rotation of the Manitoba flour glutenine solution had barely fallen to the initial value of the specific rotation of the English flour glutenine even after standing 267 hours at 37°C .

Both objections become untenable in view of the fact that similar investigations carried out on further samples of the glutenines from the same flours yielded confirmatory results. If the differences observed between the two glutenines arise from the operation of factors involved in the objections 1 and 2, then it would be a remarkable coincidence if such factors should operate with exactly equal effect in the case of the independent samples which were examined.

The results of the investigation may therefore be interpreted as demonstrating the non-identity of the glutenines from strong and weak flours.

If, as seems probable, the characteristic physical differences between the gluts of the two flours are related to the differences existing between the glutenine fractions, then it would at first sight appear feasible to produce a "strong" gluten by preparing a moist mixture of English flour gliadine with Manitoba flour glutenine, or a "weak" gluten by mixing together Manitoba flour gliadine with English flour glutenine. Attempts to demonstrate this possibility, however, met with no success,

the reasons being twofold: 1. It is not possible by grinding the proteins together to effect the same intimacy of mixture as occurs naturally in the flour. 2. The physical properties of the proteins themselves have probably been considerably modified during the process of their isolation as a result of prolonged contact with different reagents (alcohol, ether, alkali, etc.).

The view has been put forward by Kosutany⁽⁹⁾ that glutenine is derived from gliadine by the splitting off of water. The quantitative work of Osborne in connection with the hydrolysis of these proteins has shown beyond doubt that they are two absolutely distinct substances, and a comparison of the racemisation data for the glutenines with those of the gliadines confirms Osborne's view. The gliadines exhibit distinctly different optical behaviour during racemisation from that displayed by the glutenines.

SUMMARY AND CONCLUSIONS.

The gliadine and glutenine proteins from typical strong and weak wheat flours have been isolated and investigated by comparative methods.

The gliadines from the two sources have been shown to be identical proteins. This conclusion, which is in harmony with the earlier results obtained by Wood, has been arrived at on the following grounds:

1. The identity of their optical behaviour when racemised by dilute alkali at 37° C. under three different sets of conditions.
2. The identity of their specific rotations in 70 per cent. alcohol.
3. The identity of their combining capacities for alkali, as determined by titration in 80 per cent. alcoholic solution by means of $N/10$ NaOH to phenolphthalein.

The glutenines from the two types of flour have been shown to be two distinct substances, this conclusion being based on their different optical behaviour during racemisation by dilute alkali.

It is suggested that the existing ideas on flour strength require modification to include the facts recorded in this investigation. It is desirable to retain the dual conception of strength as put forward by Wood. The factor which determines the size of the loaf is most probably connected with the diastatic capacity of the flour, as was suggested by this investigator. On the other hand, the factor which determines the shape of the loaf and which appears to be directly related to the physical properties of the gluten of the flour, is possibly dependent on the particular glutenine mechanism possessed by the wheat.

The results of this investigation suggest that the strong wheat synthesizes one type of glutenine and the weak wheat a different type,

whilst wheats of intermediate strength may contain varying proportions of the two glutenines. To a certain extent also, as was demonstrated by Wood, the physical state of the gluten will be conditioned by the acidic and soluble salt content of the flour.

It is not claimed that the above explanation can be regarded as final, since there seems no particular reason to limit the number of possible glutenines to two, nor indeed is it feasible to rule out, on the available evidence, the possibility of the existence of more than one gliadine amongst the wheats. It is hoped to continue the investigation further along these lines.

It has been shown that gliadine possesses a combining weight of approximately 5000, and from this the conclusion has been drawn that the gliadine molecule contains three or a multiple of three free carboxyl groups.

It has been demonstrated that a solution of glutenine in 0.2 per cent. KOH undergoes no measurable diminution in optical rotation on standing over a long period at room temperature and that only a slow change occurs in *N*/25 alkaline solution at 37°. It thus appears probable that proteins can be extracted by means of 0.2 per cent. KOH without suffering change, provided the alkaline extracts are kept cool.

The writer would like to avail himself of this opportunity to express his thanks to Professor T. B. Wood, C.B.E., M.A., F.R.S., at whose instance this investigation was undertaken and whose advice throughout has been of material assistance. Also to Dr. A. E. Humphries, with whose help the writer was able to secure the samples of flour used for the preparation of the proteins. The sample of Manitoba flour was supplied by Messrs John White and Sons and the English flour by Messrs Soundy and Co. To both these firms the writer's thanks are due.

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ON THE USE OF ARTIFICIAL INSEMINATION FOR ZOOTECHNICAL PURPOSES IN RUSSIA.

By E. I. IVANOFF,

*Director of the Central Experimental Breeding Station for
Domestic Animals, Moscow, Russia.*

ONE of the greatest problems of Russia's present economic policy is the restoration and development of farming, and in particular, cattle-farming. The war and revolution have, together with other things, destroyed enormous numbers of cattle, horses, pigs, etc., and thoroughly undermined the meat industry, as well as the sources of supply of working animals. A decrease of 50 % below the former numbers of horses is the common state of things. The preservation of cattle-farms and studs is seldom met with, and the number of stock-producing animals is at least ten times less than formerly. The terrible drought threatens to bring into this sphere of national wealth even greater destruction. At the same time there can be no doubt that without the restoration and maintenance on a definite level of stud and cattle-farming, Russia cannot return to full economic activity. The present-day state of things demands that every effort be made, every possibility found and utilised, for increasing the number of domestic animals and for improving the methods of breeding and the breeds themselves.

Mass-breeding of domestic animals must certainly go hand-in-hand with mass-improvement of the breeds. If indiscriminate raising of animals was unprofitable before the war, at the present time, with undreamt-of prices of fodder and labour, it is certainly a loss.

The greatest obstacle in the way of a successful solution of the above problems is the shortage of progenitors suitable for stud and cattle-farms. Let us take for example horse-breeding. Before the war, the number of thorough-breds in Russia was not even 1 %, and for one stud-stallion, there were 600 mares. At present the difference between the demand and supply of valuable stallions can be expressed approximately as 1 : 3000. But the number of mares served by one stallion in the pairing season is from 10-15, seldom higher, and on an average 25-30.

Consequently, in order to provide for all the available mares stud-stallions, it would be necessary either to increase the number of the latter at least a hundred times, or to increase the productivity of the present number as many times. The former, in view of the present economic conditions, cannot be realised even to the extent of 1 % of the requirements; the latter, though not fully, yet to a considerable degree can be reached by putting systematically into wide practice the method of artificial insemination which enables more than 300 mares to be inseminated during the pairing season by one stallion, instead of 25–30. The same applies to all other live stock.

The aim of the present article is to show what has been achieved in Russia with this method and to indicate the possibilities connected with the practical side of this work.

Under the term “artificial insemination” as applied to Mammals, and in particular domestic animals, is understood the introduction, by artificial means, of the semen of the male into the vagina of the female. The seminal cells or spermatozoa can be introduced either (1) in their natural medium, i.e. in the secretion of accessory sexual glands, or (2) in an artificial medium (physiological solution, Locke’s fluid, serum, etc.). In either case the essential conditions of fertilization are the natural fundamental processes and conditions: the maturity of sexual products, for example, their viability, etc. must remain unchanged. The only difference is that in artificial insemination the fate of the secreted male sexual cells is held in the hands of man, who can divide the collected material into parts and inject it into the female sexual organs by means of instruments (catheter, syringe). Thus, in the case of Mammals, strictly speaking, it is not a method of artificial fertilization, but of artificial insemination.

The possibility of artificial impregnation by means of natural sperm was demonstrated long before my work by the famous Italian man of science, Spallanzani. The small number of carefully worked experiments on animals and lack of experience on the technical side for a long time prevented this method from gaining due importance in the practices of applied zoology.

With regard to the second method of insemination, i.e. by means of an artificial medium, the very possibility of such a method was denied by some until my works appeared. Those who wish to get acquainted with the history and technical side of the subject will find detailed information in these works and in the memoirs referred to at the end of this paper.

The practical importance of artificial insemination of domestic animals is to be found in the possibility by means of this method to utilize the seminal fluid, secreted by the male when covering the female, for the purpose of inseminating a number of other females (10-20) who are "on heat"; to combat the barrenness of females caused by various mechanical obstructions (stenosis of the neck of the womb, deflexion of the neck of the womb, polypi, etc.); to cross animals differing very greatly in size and weight; to cross various types of animals (horses with asses or zebras, cows with bisons or aurochs, etc.); to utilize the reproductive capacity of a valuable male in case of fatal injury or even death of the latter resulting from causes of a non-infectious kind. (In the latter circumstance, the seminal fluid is collected from the excised sexual glands of the male, diluted with some solution beneficial to the life of the seminal cells, and injected like natural seminal fluid into the vagina of the female.)

One of the great advantages of artificial insemination is to be found in the fact that it dispenses with the necessity of bringing a valuable male into close contact with an unknown female, as the semen can be obtained with the aid of a well-known female or one specially selected for the purpose. This circumstance is particularly important in areas where such diseases as dourine, glanders, etc. are met with.

We must also point out that in artificial insemination when the presence of trypanosomes is suspected in the sperm, the possibility of making the seminal liquid free from infection without killing the spermatozoa, has in principle been proved.

Finally, in artificial insemination the whole process takes place under the control of the microscope, which makes it possible to determine in every individual case before insemination the actual degree of mobility of the seminal cells and their number. This enables the breeder to follow and to determine the productive abilities of the male before his stud career commences, and not after, as was usually the case in natural insemination.

The greatest practical importance of the method of artificial insemination is to be found in the possibility of applying it for purposes of mass-raising of domestic animals and fullest utilization of particularly valuable males. In order to secure for this method wide practical application it was necessary to work out simple and safe technical means, to verify them on a sufficient number of animals, ascertain the number of possible inseminations from one "leap," the percentage of positive results, the strength, fecundity and working ability of the young, and convince one-

self that this method will not create an attitude of mistrust in the peasant population.

Many years were devoted to these problems—beginning with my first experiments on the Dubrovski stud-farm in 1899. The practical work was carried on in the great majority of cases on horses. On cattle, chiefly on sheep, artificial insemination was practised to a fairly large extent, but usually with a scientific purpose. Experiments were also conducted on dogs, foxes, rabbits, birds and other animals. In the following, I shall submit data only from work on horses.

In the history of the development of this work in Russia, two main stages must be noted, the first being the period of experimental preparatory work on the problem under laboratory conditions (Institute of Experimental Medicine, Zoological Laboratory of the Academy of Science), and under conditions of practical life from 1899–1909 (on the special experimental station of the Department of State Stud-Farming, in the Government of Orel, Livenski district, village of Mijnee-Dolgoe, and on the estate of Askania-Nova, in the Government of Taurida, formerly belonging to Faltzfein). Only after dealing with the fundamental problems and printing the results of the experiments, had the task of advocating this method for wide use been undertaken. The Government opened a special laboratory with a physiological section and a zoo-technical station, in Askania-Nova, attached to the Laboratory of the Veterinary Department, where from 1909 special theoretical and practical courses of study were pursued on the physiology and biology of insemination and a body of specialists, mainly veterinary surgeons, was being trained for the practical application of this method on stud-farms and pairing stations.

Preparatory experimental work was carried out apart from other animals on 579 horses, and during that period, together with problems of direct, practical interest (percentage of conceptions from artificial insemination, number of possible inseminations from one "leap," etc.) problems were experimentally investigated which have a more remote connection with stud-farm interests. As the manner of carrying out the experiments and their results were made clear in my book *Artificial Insemination of Domestic Animals*, published also in German, I shall confine myself here to a very bare outline.

The above work shows that artificial insemination enables the number of mares inseminated by one stallion during the pairing season to be increased on an average ten times, and the percentage of foaling, if the work is done correctly and under conditions usual on pairing

stations (stallion of undoubted fertility, mares healthy, pairing season about three months, condition of mares ascertained and repeated insemination effected) is higher than in natural coition with the very same stallions and reaches on an average 78 %. Single insemination, without repeated injections to follow it during the period of "heat," gives only 25 % of conceptions. Conception as a result of the mare being inseminated outside the period of "heat" is an exception. In cases of persistent barrenness, due to some anatomical irregularity in the construction of the mare's genital organs, artificial insemination is a very efficient means of bringing about conception. The technical side of artificial insemination is sufficiently simple to be used under conditions prevalent on Russian pairing stations, to say nothing of stud-farms, and can be applied not only to horses but also to other domestic animals. Artificial insemination, when the necessary precautions are taken, removes the possibility of infection. Pregnancy and delivery are normal. The young in appearance, size, strength, capacity for work and fertility do not deviate from the normal, and such broods have produced a number of winners on the race-course (Dubrovski stud-farm), and in Askania-Nova over 50 horses, produced by artificial insemination, were bought for the cavalry and artillery at highest prices. Finally, it was proved beyond doubt that the peasants are quick to learn the advantages of such a method and that its wide use all over Russia is ultimately, therefore, fairly certain.

With the establishment in 1909 of the above-mentioned laboratory (Physiological Section of the Laboratory of the Veterinary Department and Zootechnical Station in Askania-Nova) the aims of which, in addition to the investigation of scientific and practical problems of the physiology and biology of insemination, included also the popularization of the method of artificial insemination and aid for institutions and persons desirous of using this method—the possibility of acquiring fuller and wider knowledge of the method was afforded to specialists (veterinary surgeons and zootechnicians) and amateurs. A special course of lectures was given here on the physiology and biology of insemination, on problems of heredity, and practical work was done on artificial insemination of domestic animals. During the period from 1909 to 1919 over 400 persons, mainly veterinary surgeons, availed themselves of the opportunity of becoming familiar with artificial insemination. Some of these people have made practical use of this method.

The work of the physiological section, in particular the work in connection with artificial insemination, attracted attention abroad, and a number of specialists (professors and surgeons) were sent to Russia

to gain acquaintance with this method (*vide Review of the Activities of Physiological Section during 1909-1913*, p. 22).

In 1914 the work was interrupted in consequence of the army's demand for veterinary surgeons, and many of the latter had no opportunity of submitting the results of their work in 1913.

The work done in Russia represents the first attempt to use artificial insemination in mass-raising of domestic animals, an attempt, as will be seen later, by no means perfect in regard to conditions; without systematic organization or strict compliance with definite instructions. The laboratory in my charge was a purely consultative institution. As was subsequently shown, that state of things made possible a great number of very material and injurious digressions from the technical method elaborated by me, and the work was often confined to one or two visits of the veterinary surgeon to a stud-farm, once or twice a week (in the course of one, two, or three weeks) and in the majority of cases was regarded as a side-issue.

The data at our disposal refer only to the period 1909-1913 (data for the latter year are by no means complete) and were collected partly by means of a questionnaire, partly from reports of the Rural Councils. The questionnaire contained a number of questions, the answers to which should have given a clear idea of the conditions and results of the work. In dealing with the material of the questionnaire only those enquiry forms were made use of, the information of which was sufficiently full and left no suspicion as to the correctness of figures.

The above material shows the general state of things to be as follows: during the period 1909-1913 inclusive, the method of artificial insemination has been used in more than 30 governments of European Russia and in Siberia.

Even in those cases where artificial insemination was introduced, not on private initiative, but as a measure of mass-improvement of the peasants' horses (governments of Kherson, Ekaterinoslav, Taurida, Bessarabia), the organization and management of this work suffered from all kinds of irregularities: for example, at the head of affairs were often found persons without any or with second-hand knowledge of the subject, incompetent people; every worker could at his discretion introduce various changes into the organization or technical side of the work. Particularly injurious with regard to the percentage of conceptions was the idea that it is possible to carry out work successfully without regard to the absence of "heat" in the mare; also excessive simplification of the technical side, including in some cases the use, instead of index-cylinders

and pharmaceutical scales, of implements suitable only for home use. As artificial insemination of horses was included in the daily work of veterinary surgeons, often being done without any remuneration, only one day a week was often devoted to it, and sometimes the whole work did not go further than one or two visits of the surgeon. For anyone familiar with the conditions of pairing it is clear that work is impossible under such circumstances, as, apart from the method of insemination, they must lead to a considerable reduction of the percentage of conceptions, owing to the impossibility of repeating the insemination on the fresh appearance of "heat" in the mare. In other cases, the lowering of the proportion of conceptions was due to injections into the womb being repeated every 7-9 days, which was bound to cause an early abortion where conception had already set in. Further, at many stations for artificial insemination, stallions were used which were known to be unsuitable for this purpose, as in natural insemination the percentage of conceptions from them did not exceed 13 and in some cases was less than 4. It is true that, as has been ascertained, the percentage of conceptions from the same stallions with artificial insemination rose from 4 to 11, from 5 to 22, from 13 to 21, but the use of such specimens was bound to lower the general proportion of conception from artificial insemination. Finally, artificial insemination was performed mainly on mares very unreliable from the breeder's point of view, or known to be sterile.

The work was carried on chiefly on Rural Councils' pairing stations, in villages tens, and sometimes hundreds, of miles distant from town or railway station.

During the period 1909-1913 artificial insemination was performed, according to the data supplied by the questionnaire, on 6804 horses¹.

In 1909 on	3	stations	57	horses.
„ 1910 „	8	„	288	„
„ 1911 „	31	„	2285	„
„ 1912 „	41	„	3397	„
„ 1913 „	17	„	777	„

The information for 1913, as pointed out above, is by no means complete.

With regard to the normal condition of the young, freedom from infection of the sexual organs of the mares in the practice of artificial insemination, low proportion of abortions, etc., the data supplied by the

¹ The above figures refer only to European Russia.

questionnaire are of a very uniform character and confirm all the conclusions arrived at in the preliminary experimental work. Artificial insemination during the above short period succeeded in gaining the confidence of the population. In some cases the number of horses brought to the pairing station during one pairing season ran into hundreds. It has been ascertained that the number of mares fertilized during the pairing season by one stallion can average 300 and over when the method of artificial insemination is used. Sometimes the data of the questionnaire supply indications that the young born were of a superior type, or in any case perfectly sound.

Only one case of deformity is recorded out of over 2000 foals born, which proportion is quite normal.

As regards the proportion of positive results, the figures, as one could expect from the foregoing, are by no means uniform. The percentage of conceptions varies between 4 and 907 and averages about 40.

For 1909 the proportion of conceptions was	40	%.
„ 1910	40.3	%.
„ 1911	35.5	%.
„ 1912	41.4	%.
„ 1913	42.3	%.

When we look into that disparity in the results of the work of different people, we find that it was caused by those irregularities, which crept into the work, as the data of the questionnaire clearly show. For example, if we were to divide the material at our disposal into two sections, one under the heading "Artificial insemination performed on mares in period of 'heat,'" and the other "Artificial insemination performed regardless of presence or absence of 'heat,'" we should find that in the first group, where a number of other irregularities and deviations from proper conditions of work remained and only that factor of "insemination without 'heat'" was excluded, the proportion of positive results equalled 49 %, while in the second group, where that factor remains, the percentage of positive results was 28.

When we examine in both these groups the influence of the factor of duration of the pairing season, we find that when

The duration of pairing season is				The % of conceptions	
Less than one month for	1st	group		38.6	
„ „	2nd	„		19	
Over one month	1st	„		54	
„ „	2nd	„		29	

With more careful organization of the work and reliable parents, the proportion of conceptions from artificial insemination reached in the work of my pupil-practitioners not only the figures shown above (78 %), but even exceeded them, rising to 87.88 and even 90 %.

Table I.

Artificial insemination took place under the following conditions: (1) the mares were "on heat," (2) the pairing period for artificial insemination was longer than one month, (3) the complement of mares was the usual one for a stud-farm, no mare being known to be sterile, (4) the stallions were known to be reliable.

Year, government, district, station and name of person in charge of operations	No. of horses sub- jected to artificial insemination	No. of conceptions resulting therefrom	No. of horses that failed to conceive	No. of unascertained cases	% of conceptions, not counting unascer- tained cases
1912. Don Army Province, Provalski Army Stud-Farm, vet. surgeon V. Y. Kartasheff	30	17	11	2	60.7
1913. Don Army Province, Provalski Army Stud-Farm, vet. surgeon V. Y. Kartasheff	31	22	3	6	88.0
1910. Govt. of Saratoff, dis- trict and town of Kuznetzk, pairing station, vet. surgeon N. A. Zhukoff	36	25	11	0	69.4
1911. Govt. of Saratoff, dis- trict and town of Kuznetzk, pairing station, vet. surgeon N. A. Zhukoff	27	17	10	0	62.9
1912. Govt. of Saratoff, dis- trict and town of Kuznetzk, pairing station, vet. surgeon N. A. Zhukoff	10	7	3	0	70.0
1912. Govt. of Kharkoff, dis- trict of Akhtyrsk, estate of Konig Bros., Trostinnetz, assistant-surgeon Gafner ...	32	28	4	0	87.5
1913. Govt. of Kharkoff, dis- trict of Akhtyrsk, estate of Konig Bros., Trostinnetz, assistant-surgeon Gafner ...	43	39	4	0	90.7
Total	209	155	46	8	77.1

It is clear from the above table that the proportion of conceptions in artificial insemination is usually higher than that in natural pairing of the same progenitors.

The following table of results of artificial insemination of horses on the zootechnical station of Askania-Nova (1912) illustrates that fact still more clearly.

Table II.

Name of stallion	Results of artificial insemination of horses on the zootechnical station of Askania-Nova in 1912			Results of natural insemination by the same stallions in the same year		
	No. of mares subjected to artificial insemination	No. of conceptions	% of conceptions	% of conceptions	No. of conceptions	No. of mares subjected to natural insemination
Harry	11	9	81.8	40	2	5
Freiherr	7	4	57.1	50	2	4
Irkutsk	11	11	100	—	—	—
Wallenstein	4	4	100	50	4	8
Gadai-Zille	6	3	50	33.3	2	6
Total	39	31	84.6	43.4	10	23

Thus, the practical work of veterinary surgeons, carried out on some thousands of horses in various parts of Russia and generally under very primitive conditions, bears out all the fundamental conclusions of the preliminary investigations and shows the great usefulness and value of artificial insemination of domestic animals for the population.

Reports and the material of the enquiry clearly indicate that where the work was conducted more or less successfully, where the veterinary surgeon was able to devote to the artificial insemination of horses sufficient time and attention, there the number of horses inseminated during the pairing season on one station reached sometimes 850, and the majority of these in such a case were peasants' horses. In the estimation of Rural Councils (Zemstvos) that work was gaining greater and greater importance, and in the year before the war meetings and conferences were being convened for the special purpose of discussing the problems of more efficient organization of the work of artificial insemination, in connection with mass-improvement and mass-raising of stocks of horses and other domestic animals; also for the purpose of preparing a definite plan of work and publishing special instructions binding for all those doing practical work in the subject (governments of Kherson, Taurida, Bessarabia, etc.).

The interruption of the work, which was getting into stride and assuming large proportions, was due entirely to the war and revolution, and as soon as problems of organization in cattle-farming again came to the front, artificial insemination became a matter of prime importance, as shortage of procreators was the greatest obstacle in the way of all former endeavours.

Already in 1919 at one of the All-Russian conferences of zootechnicists of the Department of Cattle-farming of the People's Commissariat

of Agriculture, and later on, at the subsequent All-Russian conferences on practical horse-breeding, the question of introducing the method of artificial insemination was considered to be of immediate importance. However, lack of instruments, which were lost during the revolution, and the general economic and political situation prevented work being commenced at once. The Collegium of the P. C. A. decided to open a "Central Experimental Station for investigating the problem of raising domestic animals," and one of its fundamental practical aims was the organization of the work of artificial insemination. In 1921 the station received a great number of enquiries and requests for instruments for artificial insemination of horses from the Don Province, Kuban Province, Turkestan, governments of Voronezh, Smolensk, and other places. In some places, like the Don Province, or government of Voronezh, it was proposed to place the work of artificial insemination on a broad basis. In the Don Province a special laboratory has been opened; in the government of Voronezh there are equipped stations, but the number of instruments and suitable horses is insufficient. Owing to the accumulation of unavoidable and unfavourable circumstances, the work of artificial insemination of horses could not be commenced in the spring of this year (1922), mainly in consequence of the impossibility of obtaining in proper time instruments ordered abroad. By spring 1923 the training of a staff of specialist-technicians should be completed and a number of stations opened, in the first place, for the artificial insemination of horses.

With the practical development of this method are closely connected also its economic possibilities. The price of good progenitors should rise and cattle and stud-farming should attract the attention of the population and of capitalists who should find here a good investment. While formerly horse-breeding, for example, was in Russia by no means a profitable business, but rather a necessity or a hobby, at the present time, when a stallion during the pairing season can bring a return several times greater than its cost and leave the owner a profit of some hundreds, and sometimes thousands, of gold roubles, the advantage of breeding a good stock is obvious¹. In such circumstances there is a possibility of attracting to the work of mass-breeding and improving the stocks of domestic animals private enterprise, granting it one or another privilege and guarantee eliminating great risks. It would be possible to try to

¹ Example: expenses in connection with equipment of station for artificial insemination 200 gold roubles, cost of two stallions, 1000 g.r., upkeep of horses for four months, 200 g.r., wages to groom for four months, 200 g.r., stabling, 100 g.r., total, 1700 g.r.

Receipts: artificial insemination of 600 mares at 5 g.r. per mare; total, 3000 g.r.

create in Russia great horse and cattle-ranches. In spite of the losses during the war and revolution, there are still in Russia millions of domestic animals. The females are preserved in greater numbers and the main shortage is in males. The latter could partly be brought from abroad and partly increased in number through artificial insemination. In any case Russia, with her enormous open spaces, her extraordinary variety of climate, soil, geographical surface, her wealth of still untouched zootechnical material and the millions of her population—chiefly peasant population—provides endless opportunities for the development of all kinds of animal-breeding on a large scale, from horse-breeding to, and including, the breeding of deer and silver foxes, in the case of which artificial insemination acquires particular importance, as foxes are monogamous.

It must also be remembered that with the use of artificial insemination on domestic animals we not only gain material advantage, but also save time. Formerly, in order to create a local breed or even a considerable uniform herd, from one or another valuable progenitor, it required a period of several decades. Now, with artificial insemination, this can be achieved in a much shorter period of time, and a surplus of progeny makes possible a more careful selection.

Artificial insemination is bound in the near future to assume additional importance in the zootechnical practice in connection with the solution of two fundamental problems now being investigated, namely (1) increasing the number of females fertilized during the pairing season through artificial insemination by one male, and (2) preserving for some time and forwarding to distant places the seminal solution.

The first problem has already been to some extent solved by my experiments and the observations of my pupils, veterinary surgeons, and for practical application it needs only to be verified on a number of animals.

The second problem, theoretically quite reasonable, needs for its solution the investigation of a number of questions.

The possibility of preserving alive the seminal cells of Mammals outside the organism for many days and even weeks has been demonstrated by the work in our laboratory, as well as by other investigators; this, however, is true only of seminal cells taken, with precautions of sterilization, directly from the epididymes of the seminal gland. In this case it is sufficient to prevent them from drying up and to place them in a temperature of 1 or 2° C. But for practical purposes, it is most important to preserve the seminal cells not in the above state, but in the medium

of the secretions of the accessory sexual glands (natural sperm). In this medium there can be no question of sterilization and, what is particularly important, the seminal cells coming into contact with the secretions of the accessory sexual glands change their biological properties—acquiring a maximum of energy and dying in a comparatively short time (a few hours). Some methods of elucidating the nature of factors determining these properties are already mapped out and the solution of this problem, we are entitled to think, is only a question of time.

There remain a few words to be said on the development of the technical side of the work. In view of certain inconveniences connected with the sterilization of the sponge collecting the sperm when the male covers the female, I have substituted for the 2 % solution of sodium carbonate a solution of spirits of wine (60–65 %) in which the sponge is kept about an hour, then thoroughly cleansed with sterilized water, and finally washed several times (being repeatedly squeezed out in the press) with a sterilizing physiological solution of kitchen salt, or a 10 % solution of refined sugar. This method of sterilization has been used in practical work by veterinary surgeons since 1911. Before this innovation was recommended for actual use, it underwent a number of suitable tests and investigations in the laboratory and on the experimental station in Askania-Nova which have shown its usefulness.

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THE EFFECT OF CHANGE OF TEMPERATURE ON THE BASAL METABOLISM OF SWINE.

By J. W. CAPSTICK, O.B.E., M.A.,
Fellow of Trinity College,

AND

T. B. WOOD, C.B.E., M.A., F.R.S.,
Drapers' Professor of Agriculture.

*(From the Animal Nutrition Institute, School of
Agriculture, Cambridge.)*

INTRODUCTION.

It has long been generally accepted that an animal requires more food in cold weather than in hot weather, that, in fact and within certain limits, an animal's food requirement increases as the temperature falls. The number of precise measurements however of the effect of change of temperature on food requirement is very small, and it was with the object of extending the knowledge of this important subject that the following investigation was undertaken.

The writers' attention was directed to the subject by a paper by Armsby and Fries on "Net Energy Values and Starch Values" which appeared in this Journal in 1919¹.

In this paper Armsby and Fries point out that their Net Energy and Kellner's Starch Equivalents really measure the same thing, namely the amount of energy in feeding stuffs which is available to the animal for physiological purposes. They claim further however that this net energy only is available for maintenance, and that any excess of energy above this amount is converted at once into *heat which is of no value to the animal*.

The point will perhaps be made clearer by a concrete instance, and since figures on the subject are lacking in the case of swine, it may be permissible to supply them for a steer.

Armsby states that the basal metabolism of a 1,000 lb. steer is about six therms or 6,000 calories per day. Most European authorities agree that 14 lbs. of average meadow hay supplies a maintenance ration for a

¹ This *Journal*, 9, 182.

1,000 lb. steer. Now 14 lbs. of average meadow hay supplies about 11,500 calories of metabolisable energy and about 6,000 calories of net energy. Armsby and Fries contend that only the 6,000 calories of net energy are of service to the animal, the remaining 5,500 calories being wasted. Many European writers, amongst them one of the authors (T. B. W.), have assumed that under certain conditions the whole 11,500 calories may be of service to the steer, the 6,000 calories of net energy sufficing for physiological purposes, which presumably will not vary greatly with changes of temperature, and the balance of 5,500 calories saving the oxidation of further material to meet the increased energy required to maintain body temperature in unusually cold surroundings. It is significant to note that whilst Armsby's measurements were made in general in a calorimeter at about 65° F. or summer temperature, most European experiments on the maintenance of steers have been made in unheated stalls or sheds at winter temperatures of between 40° F. and 50° F. Such a difference in temperature, amounting to from 8 to 14° C. may possibly account for the difference of opinion between American and European investigators. It was with the hope of throwing some light on this point that the writers undertook the investigation described in the following pages. Unfortunately their calorimeter was not large enough to allow of experiments with a 1,000 lb. steer. The experiments were therefore carried out on a hog.

The investigation also deals with another point. Previous investigations on the effect of temperature on metabolism have shown that at ordinary temperatures an animal maintains its body temperature approximately constant by the rearrangement of its blood circulation. When the air temperature is low, the blood is deflected from the skin to the internal organs: the skin gets cold and loses less heat, but the internal temperature is maintained. When the air temperature is high much blood is sent through the skin where it loses much heat and cools the internal organs.

This method of regulation is however insufficient to maintain a constant body temperature when the air temperature falls below a certain point which has been called the critical temperature. Below this point, a fall in the air temperature necessitates the oxidation of further material. The basal metabolism will therefore attain a minimum at the so-called critical point. Below this point it will increase by the heat value of the extra material oxidised.

EXPERIMENTAL.

The experiments described below were carried out in the large calorimeter described by one of the writers (J. W. C.) in a previous number of this Journal¹. As the method of working is fully described in that paper nothing need be said here as to the details of the manipulation.

The experiments were made on a Large White pedigree hog bred by Mr K. J. J. Mackenzie on the Cambridge University Farm. The hog was born on January 26th, 1921, and was castrated on April 1st. The experiments began at the end of November when the hog was 10 months old and continued to the following April.

The food given to the hog was of the same general character throughout the experiments and was gradually increased so as to be roughly proportional to the two-thirds power of his weight. As an indication of the nature and amount of his food, he received when his weight was 300 lbs. the following daily ration in two meals:

2 lbs. sharps,	2 lbs. barley meal,
1½ lbs. bean meal,	1 lb. maize meal,
1 lb. fish meal.	

In the intervals between the fasting periods, which lasted from four to six days, he was kept in a small paddock in the open air with a shelter of wattle hurdles. In severe weather he was put in a sty in the laboratory. As he stripped his paddock bare of vegetation very quickly he was given a little green food each day.

The care of the hog was in the hands of Capt. J. S. Morgan who kept him in excellent condition throughout. The fact that the hog gained 157 lbs. in weight in the course of the experiments, which lasted 140 days, in spite of his being without food for about a quarter of that time, is sufficient indication that the fasts had no ill-effect on his health.

The hog was put in the calorimeter about 9 a.m. and remained there in darkness and without food, usually for five days. Regular supplies of water were introduced from outside. During this period readings were taken whenever the galvanometer curve showed that he had been asleep for a sufficiently long time to get rid of the effects of muscular activity. From these readings the heat evolution was calculated. No analyses were made of the excreta nor was the respiratory exchange observed.

After each fasting period he was given about a fortnight to recover

¹ This *Journal*, **11**, 1921, 408.

before being put in the calorimeter again. This proved to be long enough to keep him in good health.

The heat given off by the hog was measured in the usual way by observing the rise of temperature of a stream of water circulating round the calorimeter, additions being made for the latent heat of the water vapour brought out in the ventilating air, for the sensible heat brought away by the ventilating air and for leakage of heat through the walls of the calorimeter.

Details of the apparatus and the methods of measurement are given in the paper quoted above. It is sufficient to say here that the rise of temperature of the circulating water is measured by a thermoelectric couple which traces a continuous record on a Cambridge and Paul Thread Recording Galvanometer. This continuous record has proved to be a very valuable feature of the apparatus. The writers consider that it has enabled them to obtain more accurate measurements of the true Resting Metabolism than have been obtained hitherto.

The curve shows at a glance whether the hog is asleep or not, how long he has been asleep and whether his metabolism is rising or falling. The slightest movement of the hog is recorded at once and the nature of the hump on the curve often reveals its cause. An instance of this is given in Fig. 1.

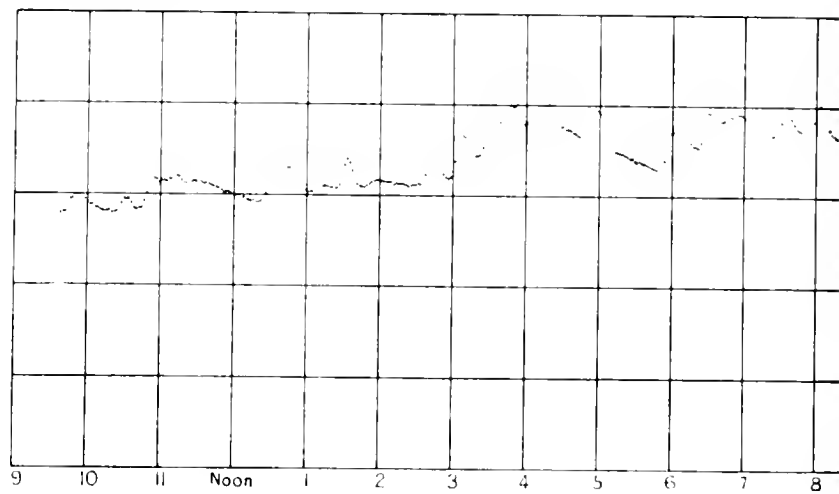
Moreover there are occasional lapses in the apparatus. An electric current may fail through a bad contact or other cause. The thermostat may strike work through a heater burning out, dirty mercury causing the relay to go out of action, etc. After some experience it has been found that all these have their characteristic effects on the curve, so that an observer watching the galvanometer can go at once to the source of the trouble and remove it before any harm is done.

In the earlier experiments the hog was very regular in his habits. He seldom slept in the day-time but kept in continual motion, his metabolism being very irregular and mounting gradually higher until about 5.30 or 6 p.m. when he went to sleep, and usually did not stir until about 6 o'clock on the following morning, except that at some time in the earlier part of the night he stood up to empty his bladder.

Fig. 1 is the curve obtained on the second day of the experiment which began on January 5. It has been chosen as showing several of the characteristic features of these curves. The irregularity of the curve in the day-time is much the same as is found in most of the curves, except on the first day of the fast when the hog often slept a part of the day.

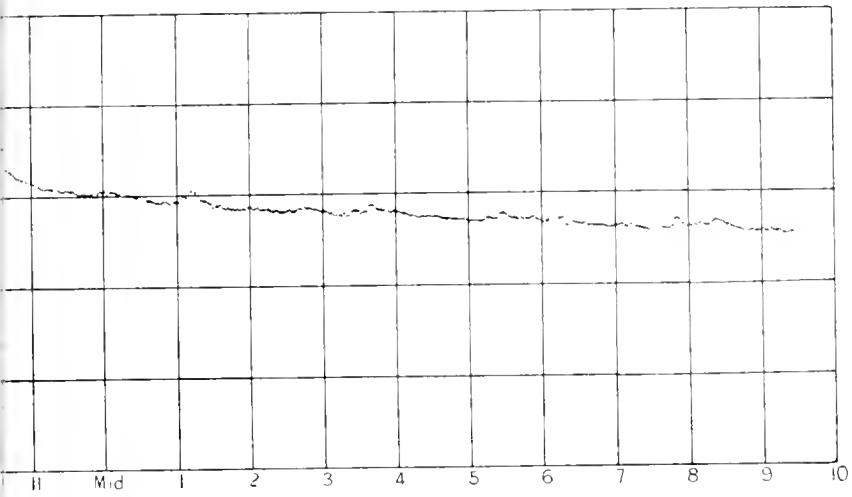
The small hump at 10.30 p.m. is due to the hog's rising to empty

Jan. 6th, 1922



between pp. 260—261

Jan. 7th, 1922



his bladder. Its shape is quite characteristic of urination. The warm urine falling on the floor of the calorimeter causes a rapid rise in the temperature of the outlet water followed by an equally rapid fall as the urine cools. This fall is checked by the rise of metabolism due to the movement of the hog, which does not show itself so quickly as the rise due to the warm urine.

The peak at 1.15 a.m. was caused by a small movement of the hog.

It is obvious that it is impossible to take any measurements of the resting metabolism in the day-time or the earlier part of the night. When the hog has gone to sleep it takes many hours for the metabolism to sink to its correct resting value uncomplicated by the effects of muscular activity. The fall during the night is not entirely due to recovery from the day's activity. It is prolonged by the lag in the galvanometer readings arising from the heat capacity of the calorimeter. This heat capacity, whilst not altering the total area of a hump, reduces its height and spreads it over a greater time. Further it is known that the body temperature in man falls to a minimum in the early hours of the morning and it is possible that there is a similar fall in swine which would in itself cause a fall in the heat evolution during the night.

The final conclusion from the study of these galvanometer curves is that it is only on rare occasions that observations of the resting metabolism free from the effect of muscular activity can be made at any other time than in the early hours of the morning.

The galvanometer records have been taken continuously through the whole period of the fast. The readings of the various thermometers, etc. needed for calculating the heat evolution have in general not been taken during the day. Through the night they have been taken at hourly or occasionally at half-hourly intervals, unless the curve showed that the readings would be useless. At the end of each day the curve and the record of the readings were carefully studied to find the time or times at which the metabolism most nearly approached a steady minimum. It is not possible to be biased in making the selection for the calculation is so long and involves so many readings that it is quite impossible to foresee the result before the calculation is completed. Most frequently only one point was selected from the day's records—occasionally two or three—on a few occasions no point showed sufficient steadiness. The metabolism was in a few cases calculated at points within 24 hours of the hog's entering the calorimeter, but these early readings have less weight than those taken later. The hog never really settled down until the second night.

Experiments have been made at a series of temperatures ranging from 10.3° C. to 23.7° C.

It is not possible to state exactly what was the temperature of the hog's surroundings. Take for instance the experiment at what we have called 13.3 in Fig. 2. The water entered the circulating pipe at 13.3 and left it at 13.5. The average temperature of the calorimeter body was therefore somewhere between these temperatures. The ventilating air entered the calorimeter at 12.4 and left at 14.4. What then was the effective temperature of the calorimeter from the point of view of the hog's metabolism? One can only make a guess. In order to have something definite the writers have adopted the temperature of the inlet water as defining the temperature of the hog's surroundings, fully realising that the actual effective temperature is probably a little different. The point is not one of any great consequence as the critical temperature is rather indefinite. It is not strictly a *temperature* but a set of circumstances of which temperature is the most important, for air circulation, humidity, etc. are not without effect.

Table I.

The time is given in hours since the last meal.

In the 23.7° experiment the earlier readings were lost through a defect in the galvanometer circuit.

Date Dec. 4 Weight 216 lbs Temp. 13.3°		Date Dec. 19 Weight 231 lbs Temp. 16.9°		Date Jan. 8 Weight 252 lbs Temp. 10.3°	
Time	Metabolism	Time	Metabolism	Time	Metabolism
14	2.927	41	2.058	19	3.188
43	2.139	67	1.869	42	2.374
66	1.926	90	1.702	66	2.227
93	1.852	114	1.725	90	2.105
116	1.833				
140	1.851				

Date Feb. 5 Weight 293 lbs Temp. 20.4°		Date Feb. 25 Weight 306 lbs Temp. 13.6°		Date Mar. 22 Weight 342 lbs Temp. 12.8°		Date Apr. 23 Weight 373 lbs Temp. 23.7°	
Time	Metabolism	Time	Metabolism	Time	Metabolism	Time	Metabolism
35	1.927	45	2.221	28	2.647	63	1.870
39	1.904	68	2.019	38	2.438	84	1.750
65	1.702	93	1.955	58	2.266		
114	1.562	113	1.936	108	2.126		
				134	2.115		

Table I shows the whole of the observations that have been made. At the head of each section is given the date on which the hog left the calorimeter, his weight at the end of the fast before receiving his first

meal and the temperature of the inlet water. Below these data is given the calculated metabolism in kilogram-calories per minute at various numbers of hours from the hog's last meal.

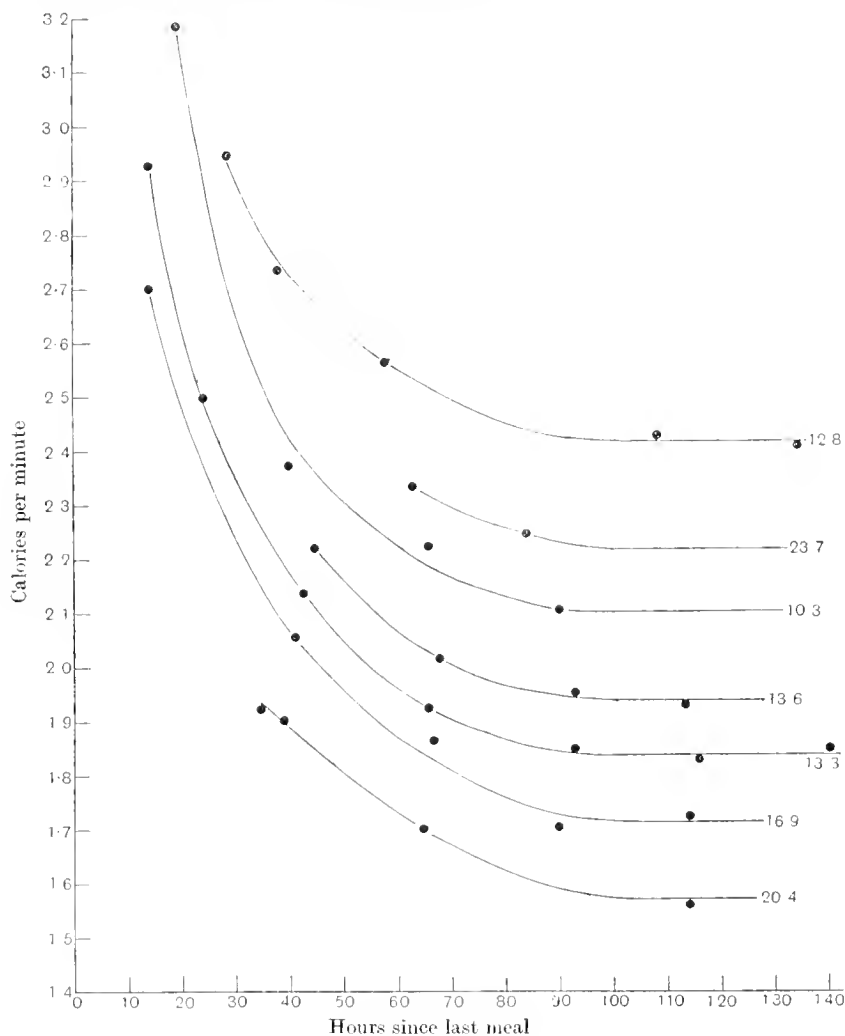


Fig. 2.

Fig. 2 shows the results in Table I plotted on a single diagram. The ordinates are calories per minute and the abscissae are hours since the last meal. The 12.8° curve is raised .3 cal. and the 23.7° curve is raised .5 cal. to keep them clear of the other curves.

It will be seen that the points fall very well on a series of similar curves. The fact that the divergences of the points from the curves are so small affords some evidence of the general accuracy of the measurements and supports the belief of the writers that they have obtained the true resting metabolism.

The curves show a very close similarity to each other as regards their shape. This similarity formed the subject of a paper read by the writers before the Royal Society. The paper has been printed in the *Proceedings of the Royal Society*¹ and nothing need be said on the matter here.

The circumstances of the experiment at 237 made it impossible to get more than two satisfactory observations and these were both taken before the hog had quite reached his basal metabolism. The basal metabolism was therefore obtained in this case by drawing a curve through the two observed points parallel to the remaining curves. This procedure seems to be justified by the conclusions reached in the paper read before the Royal Society.

Inspection of the curves shows that in every case the terminal horizontal part, which gives the basal metabolism, is reached between 90 and 100 hours after the last meal. Tangl² states that the hogs with which he worked reached their basal metabolism in 72 hours. In the present experiments there was always a perceptible fall after 72 hours. From some experiments which the writers are carrying out at present it would seem that age has an effect on the time at which the basal metabolism is reached, for a young hog weighing about 35 lbs. reaches his basal metabolism in about two days.

The basal metabolism is found graphically from the curves in Fig. 2 and is tabulated in the fifth column of Table II below.

Table II.
Summary for Critical Temperature.

Date	Age	Temp.	Weight	Basal metabolism	Reduced to 300 lbs.	Reduced to 133	Age correction to 420 days	Corrected basal metabolism
1921								
Dec. 4	312 days	13.3° C.	216 lbs	1.840	2.291	2.291	- .391	1.900
.. 19	327 ..	16.9° C.	231 ..	1.715	2.057	—	- .290	1.767
1922								
Jan. 8	347 ..	10.3° C.	252 ..	2.102	2.361	—	- .195	2.166
Feb. 5	375 ..	20.4° C.	293 ..	1.570	1.595	—	- .092	1.503
.. 25	395 ..	13.6° C.	306 ..	1.940	1.914	1.937	- .038	1.876
Mar. 22	420 ..	12.8° C.	342 ..	2.120	1.943	1.905	0	1.943
Apr. 23	452 ..	23.7° C.	373 ..	1.720	1.489	—	+ .010	1.499

¹ *Proc. R.S. B.* **94**, 35.

² *Biol. Zeitsch.* **44**.

Before the measurements can be used for finding the critical temperature they must be corrected to a standard weight and a standard age.

The standard weight chosen is 300 lbs., as this lies almost half-way between the extremes of weight. In making the correction it is assumed that the metabolism is proportional to the hog's surface area and that the area is proportional to the two-thirds power of the weight.

The basal metabolisms in the sixth column of Table II are calculated from those in the fifth column on these assumptions.

In order to provide data for a possible age correction observations of the basal metabolism were made in the neighbourhood of 13° C. on December 4, February 25 and March 22. These three were at 13·3°, 13·6° and 12·8° respectively. The difference is not great, but as they fall at a part of the range where the change of metabolism with temperature is considerable they should be corrected to the same temperature before they are used for finding the age correction.

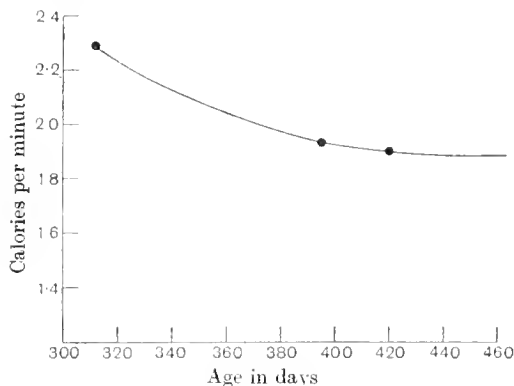


Fig. 3.

This correction requires a knowledge of what it was the object of the experiments to find—namely, the relation between basal metabolism and temperature—and it is somewhat illogical to make such a correction at this stage. As however the correction is quite small it is not likely that any appreciable error will be made by assuming for this purpose that there is a linear relation connecting the metabolism and temperature between 10·3° and 20·4°. This gives a fall of ·077 calories per Centigrade degree, and using this value we have the corrected metabolisms shown in the seventh column of Table II. The final curve of Fig. 4 shows that a linear relation is not far from the truth.

Fig. 3 shows the result of plotting the basal metabolism at 13·3°

against the hog's age in days. It appears that at the end of the experiments age had almost ceased to have any effect.

We have next to correct all the metabolisms in the sixth column of Table II to some one date. It is immaterial what date is chosen. The writers have selected March 22 when the hog was 420 days old.

The corrections to be added or subtracted are given in the eighth column of Table II. In making these corrections it has been assumed that the effect of increasing age on the curve connecting metabolism and temperature is to cause it to move bodily downwards always keeping the same shape. It may be that it would be more correct to assume that the various points on the curve move downwards in the same proportion.

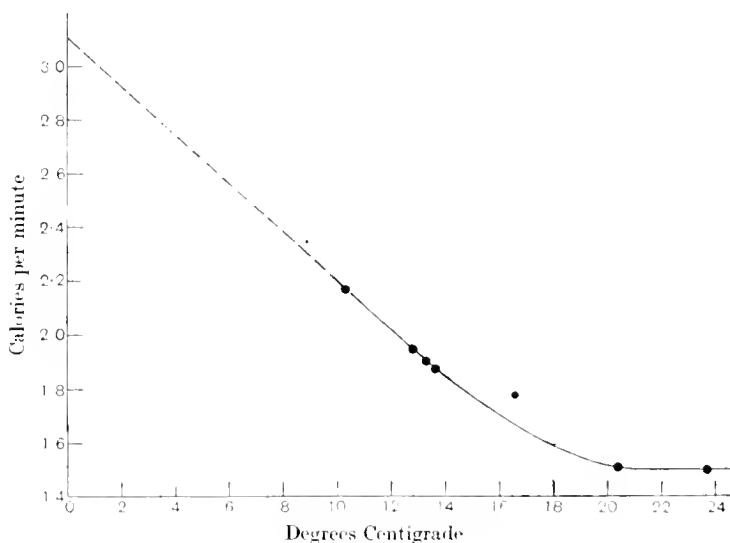


Fig. 4.

The writers see no adequate theoretical reason for preferring either of these methods to the other. For the present purpose it makes very little difference which method is used. The point might be worth investigating experimentally, but it would be difficult to secure the necessary accuracy.

We have finally the corrected values of the basal metabolism shown in the last column of Table II and plotted against temperature in Fig. 4.

The points fall very well on the curve with the exception of that at 16.9° which is 6½ per cent. too high. The writers are unable to account for

this anomaly except on the grounds that the experiment at 16.9° was unsatisfactory throughout. The hog was scarcely ever really quiet throughout the whole fasting period with the result that the galvanometer curve was less regular than usual. It should be noted that errors due to the hog would almost certainly cause the observed metabolism to be too high, whilst experimental errors would be indifferently high and low. There was also an instrumental failure on the last day of the 16.9° experiment. One of the electrical heaters in the thermostat burnt out and there was a great disturbance of the curve before the fault could be remedied.

The remaining points however are sufficiently consistent to enable the writers to state that the critical temperature is very near to 21° C.—remembering, however, what has been stated above, that the real average temperature of the hog's surroundings may be somewhat different.

This conclusion agrees very well with that reached by Tangl who states that he found the critical temperature to be between 20° and 23°.

The actual metabolism at the critical temperature is 1.50 calories per minute for a 300 lb. hog or 2,160 calories per day.

The exact relation between a hog's surface area and his weight is not known. Tangl accepts Voit's formula $A = 9.02 W^{\frac{2}{3}}$. This gives 904 calories per day per square metre, which is near the value generally adopted for human beings.

The values of the basal metabolism at different temperatures have a practical interest for pig breeders as they enable us to calculate the maintenance ration at various temperatures.

Table III.

Temp.	Basal metabolism of a 300 lb. hog	Temp.	Basal metabolism of a 300 lb. hog
0	3.10	12	2.01
2	2.92	14	1.84
4	2.74	16	1.70
6	2.56	18	1.59
8	2.38	20	1.51
10	2.19	22	1.50

Table III gives the metabolism of a 300 lb. hog at intervals of 2° from 0° C. to 22° C. The metabolism between 10° and 20° is taken from the full curve in Fig. 4. Actual observations of the metabolism could not be made at very low temperatures as the temperature of the town supply water did not permit of the calorimeter being set to anything below 10°. In order to get an estimate of the metabolism below 10° it is therefore necessary to use the uncertain expedient of extrapolation.

As the observed curve is nearly a straight line below about 16° it has been continued backwards in a straight line to 0, the dotted line being the part of the curve obtained by extrapolation. The values of the metabolism in Table III are taken from this extended curve.

It may be presumed that in so far as the extrapolated values are in error, the error is on the side of their being too low since the observed part of the curve is concave upwards. They will however provide a sufficient approximation for our present purpose.

It will be seen that the maintenance ration at 0° is more than double that at 22° , and both these temperatures are not unfrequently met with on farms in this country.

CONCLUSIONS.

The critical temperature of the hog under experiment was approximately 21°C .

At this temperature his basal metabolism was a minimum and amounted to 2,160 calories in 24 hours when he was 420 days old and weighed 300 lbs. This corresponds to 904 calories per day per square metre of body surface.

As the temperature of his surroundings fell below 21°C ., the basal metabolism increased at the rate of about 4 per cent. per degree Centigrade, which corresponds to an increase of about 40 per cent. for a temperature difference of 10°C . which is commonly found between summer and winter conditions.

Thus, if the same law holds in the case of a steer whose basal metabolism at 18°C . or summer temperature is 6,000 calories, his basal metabolism at 8°C . in an open yard in winter would be 9,000 calories.

The suggestion is that the increase of 3,000 calories is met by the utilisation of the thermic energy of the coarse fodder included in his ration.

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THE FUNGICIDAL PROPERTIES OF CERTAIN SPRAY-FLUIDS. III.

By E. HORTON AND E. S. SALMON,

Research Department, S.E. Agric. College, Wye, Kent.

As an aid to the elucidation of the problem of the exact fungicidal value of a mixture of lime-sulphur and arsenate of lead—a matter of great importance to the practical fruit-grower—spraying experiments were carried out during 1921 with certain spray-fluids containing arsenic or lime-sulphur and its constituents.

Method. In order to ascertain within narrow limits the fungicidal value of any solution, it is obviously necessary to maintain as fixed a biological standard as possible. To ensure this, the fungus used in comparative experiments should be in the same stage of development and, if it is a parasite, the host-plant used should also be “standardised” as far as possible—since it has been shown⁽²⁾ that the same stage of a fungus may be more easily killed when on the older leaves of a plant than on the younger.

In all the experiments described below, the fungus used was *Sphaerotheca Humuli* (DC.) Burr., and the stage selected for spraying was the young, “powdery,” conidial stage produced on young leaves, at the 3rd to 9th node, of rooted cuttings of hop-plants (*Humulus Lupulus*, Linn.) grown in an unheated greenhouse. To escape as far as possible variation on the side of the host-plant, with possible consequent effects on the vigour of the parasitic fungus, all the plants used were clone-plants, i.e., plants raised vegetatively by cuttings taken from one individual hop-plant. The general methods of spraying and of the examination of the sprayed leaves, etc. have been described in previous articles (1, 2). In order to secure complete wetting of the fungus, calcium caseinate (1 per cent.) was added to the solutions used.

Where care is taken to obtain in this way strictly similar biological conditions of parasitic fungus and host-plant, it becomes possible, as shown below, to determine within narrow limits the fungicidal value of a solution. In all the experiments described below “powdery” conidial

270 *Fungicidal Properties of Certain Spray-Fluids.*

patches of *S. Humuli* were selected for spraying on one leaf at a node, while the similar patches of mildew on the other leaf at the same node served as "controls."

The experiments carried out fall into two classes: viz. (1) those in which arsenical solutions were used; and (2) those in which lime-sulphur or its constituents were used.

1. EXPERIMENTS WITH SOLUTIONS CONTAINING ARSENIC.

Several investigators have stated from time to time that arsenic possesses some fungicidal power. Waite(3), who appears to have been the first to have discovered this, stated in 1910 that arsenate of lead "seems to possess considerable fungicidal value, though probably not enough to be depended upon for general use." Similar statements have been made by Clinton(4) and Watkins(5). Wallace, Blodgett and Hesler(6) considered that arsenate of lead was "about as effective as lime-sulphur" in controlling apple-"scab" (*Venturia inaequalis*) in the orchard; but state that in laboratory experiments with germinating spores of apple-"scab," "brown rot" and *Sphaeropsis*, arsenate of lead was found to have only "a weak fungicidal value." Morse has repeatedly claimed fungicidal powers for arsenate of lead; in 1911 he stated(7) that "arsenate of lead paste controlled apple scab as well as Bordeaux mixture and lime-sulphur," and, from observations made on trees in sprayed orchards, that "even small or medium applications of arsenate of lead possess a distinct fungicidal value"; in 1915, the conclusion was drawn(8), again from observations made in sprayed orchards, that "arsenate of lead was less efficient in controlling scab than the standard fungicides, but still noticeable." In 1916 Morse stated(9) that arsenate of lead alone controls apple-"scab" on the fruit (apple) as well as, or better than, lime-sulphur mixed with arsenate of lead, but added that "laboratory experiments by Mr M. Shapovalov failed to show for arsenate such high fungicidal properties as the field experiments indicated. Germination of conidia of the fungus (*Venturia inaequalis*) placed in similar dilutions of the poison was reduced and retarded, but by no means prevented." In 1918, Morse, in recording(10) the results of spraying experiments in apple orchards, stated that "the use of arsenate of lead alone as a spray reduced the amount of scab on the foliage from 90 to 95 per cent." Sanders, in 1917, from spraying trials made in the orchard, recorded(11) that "the arsenate of lime alone seems to be almost as valuable a fungicide as the arsenate of lead alone." On the other hand, the statement has been made by Pickett(12) that arsenate of lead has practically no fungicidal value.

As will be seen from the above, the statements as to the fungicidal value of arsenic rest on observations made in the field, except in two cases where experiments were made with germinating spores. We have not been able to find in the literature of the subject a single case in which the experimenter has used arsenical solutions of varying strengths on a fungus kept under close observation and determined the necessary strength for complete fungicidal action.

Materials used. Sodium arsenates. In the first instance the sodium hydrogen arsenate Na_2HAsO_4 and trisodium arsenate solutions were prepared by neutralising a solution of a known weight of pure arsenic acid with the theoretically necessary weights of sodium hydroxide (Experiments 23, 25, 27). Afterwards pure $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ was prepared by crystallising the commercial dodecahydrate above 20° , and the trisodium arsenate was prepared from it by treating the solution with the calculated weight of sodium hydroxide solution and crystallising. The amount of arsenic in these salts was estimated by distilling with cuprous chloride and hydrochloric acid and titrating the distillate with iodine (Experiment 41).

Calcium Arsenates. In the first instance sprays of the required concentration of these substances were prepared simply by taking a solution of the corresponding sodium salt of double the required strength and diluting to double the volume with a solution of the necessary amount of calcium chloride (Experiments 19, 20, 22, 28). The spray solution of course contained sodium chloride (which at so great a dilution was probably quite without effect on the hop leaf) as well as the calcium arsenate. In this method of preparation (with dilute solutions) calcium chloride produces a precipitate with sodium hydrogen arsenate but the precipitate dissolves on dilution; with trisodium arsenate the precipitate is insoluble.

For later experiments two calcium arsenates were prepared, the first by treating a solution of 11.5 grams of calcium chloride in 25 c.cm. of water with a solution of 32 grams of crystallised disodium hydrogen arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$), the second by adding a solution of calcium chloride of the same strength to a solution of 31 grams of crystallised disodium hydrogen arsenate which had been previously treated with a solution of 4 grams of pure sodium hydroxide. In each case the precipitate was filtered on a Buchner funnel, thoroughly washed with cold water and dried in the air at room temperature. For the purpose of spraying the calculated quantities of these arsenates were weighed out, triturated with water and the suspension diluted (partly with 10 per cent. calcium caseinate solution) to the required strength (Experiments 31, 33, 34).

272 *Fungicidal Properties of Certain Spray-Fluids*

Experiment 23. Disodium arsenate (containing 0.096 per cent. As_2O_5). On the first day (24 hours) after spraying, all the mildew-patches on all the sprayed leaves were barren and apparently dead, while those on the control leaves were very vigorous and densely "powdery." By the third day after spraying, the fungicidal nature of the solution was clearly evident. Each spot of mildew was conspicuous as a dead, white mycelial patch. There was also a brown patch of dead leaf-cells, sometimes corresponding in size with the area of the dead mycelium, sometimes smaller. The healthy parts of the leaf showed no trace of injury.

Experiment 27 bis. Disodium arsenate (0.024 per cent. As_2O_5).

Experiment 41. Disodium arsenate (0.02 per cent. As_2O_5).

Both the above solutions proved fungicidal; no leaf-cells underlying the mildew-patches were killed by the solution.

Experiment 25. Trisodium arsenate (containing 0.077 per cent. As_2O_5). The fungicidal effect of the solution was clearly evident 24 hours after spraying. By the third day, on some of the leaves, small brown, "burnt" patches of leaf-cells, underlying the mildew-patches, had appeared, similar to those noticed in *Experiment 23* (see above).

Experiment 20. Dicalcium arsenate (0.096 per cent. As_2O_5). By the fourth day all the mildew-patches on the sprayed leaves were dead; those on the "control" leaves (sprayed with 1 per cent. calcium caseinate) were very vigorous and densely powdery. No trace of "scorching" occurred on the sprayed leaves.

Experiment 22. Dicalcium arsenate (0.018 per cent. As_2O_5). On the first day (24 hours) after spraying, the mildew-patches on all the sprayed leaves (four) were dead—the mycelium, although still white, was composed of *hyphae* in a floccoso-collapsed condition. All the mildew-patches on the "control" leaves (four), which had been left unsprayed, were very vigorous and densely "powdery."

Experiment 28. Dicalcium arsenate (0.024 per cent. As_2O_5). On the second day after spraying most of the mildew-patches were barren—a very few patches, however, bore a few weak *conidiophores* at their edges. The mildew-patches on the "control" leaves (unsprayed) were all very vigorous and densely "powdery." By the seventh day no further growth of the mildew had taken place. By the thirteenth day the mildew-patches were all killed; on three leaves there were small, distinct, "scorched" areas of dead leaf-cells limited to the places where the mildew had occurred (cf. *Experiment 23*). The general appearance of the sprayed leaves suggested that the solution used was just fungicidal.

Experiment 33. Dicalcium arsenate (0.01 per cent. As_2O_5). The solu-

tion exerted a slight checking action on the mildew-patches on two of the five sprayed leaves; on the remaining three leaves the mildew-patches, on the fourth day after spraying, were as "powdery" as those on the "control" leaves (unsprayed). The solution at this strength, then, was practically non-fungicidal.

Experiment 19. Tricalcium arsenate (0.076 per cent. As_2O_5). By the fourth day after spraying the mildew-patches on all the sprayed leaves (five) were barren and apparently dead; on the "control" leaves (five), sprayed with 1 per cent. calcium caseinate, all the mildew-patches were very vigorous and densely "powdery." By the sixteenth day it was clear that the solution had been completely fungicidal. No trace of "scorching" appeared anywhere on the leaves.

Experiment 31. Tricalcium arsenate (0.02 per cent. As_2O_5). By the third day several of the mildew-patches on four of the (seven) sprayed leaves had developed fresh *conidiophores* at the edges of the patches—a clear indication that the solution was not quite fungicidal; on three of the leaves the patches were all barren. At this date all the mildew-patches on the seven "control" leaves, which had been sprayed with 1 per cent. calcium caseinate, were very vigorous and densely "powdery." At the end of the experiment, i.e., on the twelfth day, the condition of the mildew on the sprayed leaves was as follows: leaf (1), (2), (3), at the 4th, 5th, 6th nodes; clustered *conidiophores* round the edges of all the patches, while the *mycelium* was barren and probably dead at the centre of each patch. This condition indicates that the solution used was of a not quite fungicidal strength. Leaf (4) at the 3rd node, and leaf (5) at the 5th node; the mildew-patches now bearing densely clustered "powdery" *conidiophores* at their edges—indicating that the solution was almost non-fungicidal. Leaf (6) and (7), at the 6th and 7th nodes: many of the patches remained permanently barren and were probably killed; a few developed weak scattered *conidiophores* at their edges. Here the solution proved almost but not quite fungicidal. In previous experiments (2) it had been found that a "powdery" patch of mildew is easier to kill when growing on an older leaf than on a younger leaf.

Experiment 34. Tricalcium arsenate (0.01 per cent. As_2O_5). By the seventh day only a slight checking action on the growth of the mildew was evident, most of the patches now bearing densely clustered, more or less "powdery" *conidiophores*. It was clear that the solution at this strength is practically non-fungicidal.

2. EXPERIMENTS WITH "LIME-SULPHUR" AND ITS CONSTITUENTS.

These experiments preliminary to a contemplated study of the fungicidal and insecticidal properties of a mixture of lime-sulphur and arsenates were for the purpose of ascertaining to which constituent or constituents of lime-sulphur the fungicidal property of this spray-fluid is due, and also to determine the exact strength of such constituents fungicidal for the "powdery" conidial stage of *S. Humuli*.

It was found⁽²⁰⁾ that the addition of a solution of calcium caseinate—a substance first used with a fungicide, apparently, by Vermorel and Dantony⁽¹³⁾—increased the wetting powers of lime-sulphur so satisfactorily that its fungicidal properties at various dilutions could be accurately measured. Concordant results were obtained when a solution of lime-sulphur of a certain strength, to which a solution of calcium caseinate had been added, was compared, using the method described below, with a lime-sulphur solution alone of the same strength. Some of the details of these experiments may be given here, since they serve also to show the close approximation of the lower limit of the fungicidal concentrations and the upper limit of the non-fungicidal concentrations of lime-sulphur for the particular fungus.

The method employed was—since a soap solution cannot be used with lime-sulphur for chemical reasons—first to spray the mildew with a 1 per cent. soft soap solution (which removed the air entangled among the *conidia* and *conidiophores* and wetted all the parts), then to spray thoroughly with water to remove the soap solution, and immediately afterwards with the lime-sulphur solution. Observations showed that the treatment with soft soap and then with water had no deleterious effect on the mildew, since by the fourth day after treatment (and often earlier) the sprayed mildew-patches were fully as vigorous and as powdery as the unsprayed ones on the "control" leaves.

In the following two experiments the lime-sulphur solution was applied after the preliminary treatment described above. A commercial brand of lime-sulphur ("Sulfinette") of 1.30 sp. gr. and containing 16.57 per cent. of polysulphide sulphur was used.

Experiment 10. Lime-sulphur, 1 part to 99 parts of water (0.16 per cent. polysulphide sulphur). The solution proved completely fungicidal.

Experiment 9. Lime-sulphur, 1 : 199 (0.08 per cent. polysulphide sulphur). On the fourth day after spraying the mildew-patches were all barren. By the sixth day scattered *conidiophores* had appeared from some of the patches, while the remaining patches were still barren. These

conidiophores persisted to the end of the experiment (14 days). Lime-sulphur at this strength (0.08 per cent. polysulphide sulphur) appeared to be just breaking down in fungicidal efficiency.

In the corresponding experiments the same brand of lime-sulphur was used mixed with a solution of calcium caseinate.

Experiment 14. *Lime-sulphur*, 1 : 99 (0.16 per cent. polysulphide sulphur) and 1 per cent. calcium caseinate. The solution proved completely fungicidal within 24 hours after spraying; and the same was the case when 0.5 per cent. calcium caseinate was used.

Experiment 15. *Lime-sulphur*, 1 : 149 (0.11 per cent. polysulphide sulphur) and 1 per cent. calcium caseinate. Complete fungicidal action resulted.

Experiment 16. *Lime-sulphur*, 1 : 199 (0.08 per cent. polysulphide sulphur) and 1 per cent. calcium caseinate. On the tenth day after spraying, a few of the mildew-patches had produced fresh, clustered *conidiophores*, and others, scattered *conidiophores*; many of the patches were dead. It was clear that at this strength the solution was not quite fungicidal.

A reference to the literature of the subject seemed to show that chemists were not in complete agreement as to what chemical compounds constitute the spray-fluid universally known as lime-sulphur (compare Van Slyke, Hedges, and Bosworth⁽¹⁴⁾; Tartar⁽¹⁵⁾; Ramsay⁽¹⁶⁾; Thompson and Whittier⁽¹⁷⁾; Bodnar⁽¹⁸⁾; Chapin⁽¹⁹⁾). The following compounds, which are known or suspected constituents, were tried singly: calcium sulphate, sulphite, thiosulphate, hydroxyhydrosulphide and polysulphide (probably pentasulphide). The result of each experiment is given below.

Materials used. *Calcium sulphate.* A saturated solution of this salt was prepared by neutralising a boiling solution of 2 grams of pure sulphuric acid in 250 c.cm. of water with a slight excess of carefully purified precipitated calcium carbonate, filtering the liquid and allowing the filtrate to cool. The spray was prepared by mixing the filtrate with enough calcium caseinate solution to give a 1 per cent. concentration of the latter.

Experiment 32. *Calcium sulphate* (saturated solution) and 1 per cent. calcium caseinate. Clustered *conidiophores* were re-formed on all the mildew-patches by the second day, and by the fourth day the patches on the sprayed leaves were as "powdery" as those on the "control" leaves.

Calcium sulphite. A solution of 25 grams of crystallised sodium sulphite in the minimum amount of water was added to a solution of

276 *Fungicidal Properties of Certain Spray-Fluids*

23 grams of calcium chloride (prepared from twice reprecipitated calcium carbonate) in a small volume of water. A white flocculent precipitate formed which quickly became powdery. This was filtered on a Buchner funnel, thoroughly washed with cold water and whilst still wet was transferred to a beaker with 250 c.cm. of water and stirred mechanically for an hour. The solid was then allowed to settle and the supernatant liquid filtered. For spraying 90 c.cm. of this solution were mixed with 10 c.cm. of calcium caseinate solution (10 per cent.).

Experiment 36. Calcium sulphite (saturated solution) and 1 per cent. calcium caseinate. Exactly the same result was obtained as in Experiment 32, described above.

A saturated solution of calcium sulphite having proved to be non-fungicidal, a suspension of the solid sulphite was adjusted (by means of titration with standard iodine solution) to contain 5 grams in 90 c.cm. of liquid and this 90 c.cm. treated with 10 c.cm. of 10 per cent. calcium caseinate solution for spraying.

Experiment 40. Calcium sulphite (suspension), 5 per cent., and 1 per cent. calcium caseinate. Exactly the same result was obtained as in Experiment 32, described above.

Calcium thiosulphate. The preparation of a small quantity of the pure salt is rendered difficult by its high solubility in water, and an attempt to obtain it by double decomposition of calcium chloride with sodium thiosulphate failed through the inferior solubility of sodium chloride. By stirring a mixture of 27 grams of pure barium thiosulphate ($\text{BaS}_2\text{O}_3 \cdot \text{H}_2\text{O}$) and 16 grams of pure calcium sulphate (CaSO_4) in 250 c.cm. of water for 10 hours and then filtering, a solution was obtained which gave a reaction for sulphate but not for barium and on titration with standard iodine solution proved to contain 1.321 grams of thiosulphate sulphur per 100 c.cm. This solution contained only calcium thiosulphate and calcium sulphate and the latter had been proved non-fungicidal. Ordinary commercial lime-sulphur solution contains roughly 1.5 per cent. of thiosulphate sulphur per 100 c.cm., so that the wash obtained by diluting with 29 parts of water contains 0.05 per cent. of thiosulphate sulphur. A spray solution containing the same amount of sulphur (as calcium thiosulphate) was prepared by diluting 3.78 c.cm. of this calcium thiosulphate solution with water, adding 10 c.cm. of 10 per cent. calcium caseinate solution and making up to 100 c.cm. This was applied (Experiment 38) and proved to be quite non-fungicidal.

A solution ten times as strong was prepared by adding 10 c.cm. of calcium caseinate solution (10 per cent.) to 37.85 c.cm. of the calcium

thiosulphate solution and diluting to 100 c.cm. This was applied (Experiment 35) and some fungicidal action was observable. The patches on the sprayed leaves were all barren on the second day after spraying; by the fourth day *conidiophores* had begun to be developed from some of the patches, and by the seventh day clustered *conidiophores* either covered these patches or had developed round their edges. The solution thus proved ultimately non-fungicidal.

Calcium hydroxyhydrosulphide. A concentrated solution of calcium hydrosulphide was prepared in the manner described by Divers and Shimidzu (*Chem. Soc. Trans.* 1884, **45**, 272) by bubbling hydrogen sulphide through a suspension of pure calcium hydroxide in water, fresh lime being added at intervals as the liquid becomes clear. A pale yellow solution was obtained which was diluted for analysis and use. The monosulphide sulphur was estimated by Chapin's method.

Commercial lime-sulphur solution (concentrated) contains about 6 per cent. of monosulphide sulphur. Hence a wash diluted to the extent of 1 in 150 (which we found to be the minimum fungicidal strength for hop-mildew) will contain 0.04 per cent. of monosulphide sulphur. Accordingly spray solutions (each containing 10 c.cm. per 100 c.cm. of the 10 per cent. calcium caseinate solutions) were prepared by diluting this calcium hydrosulphide solution so as to contain the required amount of monosulphide sulphur.

Experiment 27. 0.04 per cent. monosulphide sulphur and 1 per cent. calcium caseinate. The solution was non-fungicidal; *conidiophores* were re-formed 24 hours after the spraying, and the patches were densely "powdery" by the fourth day.

Experiment 30. 0.34 per cent. monosulphide sulphur and 0.5 per cent. calcium caseinate. The solution was non-fungicidal; the patches bore fresh *conidiophores* by the second day, and became "powdery" soon afterwards.

Experiment 29. 0.85 per cent. monosulphide sulphur and 0.5 per cent. calcium caseinate. On the second day after spraying, the patches were practically obliterated by the deposit from the spray-fluid and were barren. By the fifth day *conidiophores* had begun to develop from most patches (chiefly from their edges), even where the deposit had completely covered the patch. By the ninth day it was quite evident that the solution was non-fungicidal, most of the patches being as powdery as those on the "control" leaves.

Calcium polysulphide. Calcium hydrosulphide solution was prepared as described above and the monosulphide sulphur estimated. A measured

278 *Fungicidal Properties of Certain Spray-Fluids*

portion of the concentrated solution was heated on a water-bath with excess of powdered sulphur until no more dissolved, when the red liquid was decanted and preserved. The polysulphide sulphur in this solution was estimated by Chapin's method.

Commercial lime-sulphur solution contains about 25 grams per 100 c.cm. of polysulphide sulphur and thus gives 0.33 per cent. of polysulphide sulphur in a 1 in 75 dilution. The calcium polysulphide solution was diluted to contain 0.33 per cent. of polysulphide sulphur.

Experiment 43. Calcium polysulphide (0.33 per cent. polysulphide sulphur) and 1 per cent. calcium caseinate. The solution proved fungicidal, the fungicidal effect being evident by the fourth day after spraying.

The experiments described above tend to show that the only constituent of lime-sulphur solution which has appreciable fungicidal power (at the strength at which this solution is used in practice) is calcium polysulphide.

This result is in agreement with the previous work^(1,2) with ammonium polysulphide solutions.

SUMMARY.

The following solutions were tested with respect to their fungicidal properties towards the "powdery," conidial stage of *S. Humuli* on young hop-leaves in the greenhouse:

(1) Disodium arsenate, containing 0.096 per cent. As_2O_5 proved fungicidal and killed also patches of leaf-cells underlying the mildew-patches, but did not otherwise injure the leaf. A solution containing 0.02 per cent. As_2O_5 was fungicidal without killing any leaf-cells.

(2) Trisodium arsenate containing 0.077 per cent. As_2O_5 proved fungicidal.

(3) Dicalcium arsenate, containing 0.048 per cent. As_2O_5 proved fungicidal; containing 0.021 per cent. As_2O_5 the solution was apparently just fungicidal, but with 0.01 per cent. As_2O_5 the solution was practically non-fungicidal.

(4) Tricalcium arsenate containing 0.076 per cent. As_2O_5 is fungicidal; containing 0.02 per cent. As_2O_5 it possesses some fungicidal value, but with 0.01 per cent. As_2O_5 it is practically non-fungicidal.

(5) The following constituents of lime-sulphur wash proved non-fungicidal: calcium sulphate, sulphite, thiosulphate, hydroxyhydro-sulphide.

(6) Calcium polysulphide, at a strength of 0.11 per cent., proved fungicidal.

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THE SUGARS AND ALBUMINOIDS OF OAT STRAW.

BY S. H. COLLINS AND B. THOMAS.

Agricultural Department, Armstrong College, Newcastle-on-Tyne.

THE newer knowledge of nutrition shows that cereals and seed products are deficient in calcium, sodium, chlorine, and unknown substances, called fat-soluble *A* and water-soluble *B* sometimes referred to as "vitamines" or "accessory food factors."

McCollum(1) in America, has gone the length of proving by actual experiment that cows and their calves can be raised to perfection on nothing but the complete maize plant, although maize grain is well known as a very incomplete food. In spite of his demonstration, and in spite of the obvious fact that nothing could be more like grass than an entire cereal plant and therefore suited to herbivora, very few practical or theoretical agriculturists recognise that straw is the most likely thing in the world to correct for the deficiencies of grain feeding. The difficulty is to get straw that is eatable. The practical farmer, when he happens to get a good sample, accepts it as a gift of fate and is content to turn it into profit for himself as soon as he can. The object of the enquiry, or rather the series of enquiries of which this forms a part, is to adopt the more scientific mode of procedure and endeavour to find out what differences of feeding value naturally occur in oat straw, and which of the conditions needed for high feeding value could be repeated at will, and what light such investigation threw on the old question of why farmers in some districts can fatten cattle on swedes and straw whilst in other districts it is found impossible. Oat straw is plentiful in this country and is probably well supplied with the so-called food accessories, that is the things that the grains lack; the problem at issue is how to get more of the eatable kind and less of the uneatable kind. Provided that the straw is eaten in fair quantity, the possible diminution of growth power, by partial destruction of vitamins due to keeping straw in the stack, is of no practical importance because of the large amount of the straw.

The first subject attacked was the sugar content, but it was found during the progress of the investigations that the albuminoids were

equally promising as a subject of enquiry. It is worthy of note that whereas the chief digestible carbohydrate of oat grain is starch, which is condensed dextrose, the sugar of the straw is chiefly laevulose. In other words, straw supplies the feeding deficiency of oat grain, if cane sugar, which is a condensed form of both sugars together, is considered the complete food sugar.

Samples of oat straw from many parts of Great Britain have been examined for their chief constituents and as far as possible the conditions under which the straw was grown have been recorded. From the results obtained, averages of groups of results are given in the following notes. The general method of analysis follows common practice for oil, albuminoids, fibre and ash. The estimation of laevulose has been described by one of us(2), the estimation of the other sugars being by Fehling's volumetric method. At times the method of Ling(3) was used for an end point but in most cases the solutions were sufficiently free from colour to render Ling's indicator superfluous. At times the oat straws were extracted with ether to remove some substances that prevented the cuprous oxide from settling(4) but in most cases these precautions proved unnecessary. Nevertheless the authors consider that both methods are very valuable at times when difficulties arise. As regards Ling's indicator the authors found that a simpler recipe worked even better. A small ball rolled up from about half a yard of flower wire was placed in a test tube with 2 c.c. hydrochloric acid and 2 c.c. water and boiled for one minute. A small spoonful (about $\frac{1}{2}$ gm.) of ammonium sulpho-cyanide was added and allowed a minute or two to stand and cool. This indicator kept, in its original test tube with the residue of the iron wire, very well for a week or two, since fresh iron dissolved as fast as oxidation took place. Also carbon oxysulphide is formed which acts as a reducing agent. In practice it is better to make fresh indicator daily. In early samples pentoses were looked for, but either none or the merest traces could be found. From the polarimetric readings and other properties the unidentified sugar appears to be mostly dextrose.

The albuminoids are the usual $N \times 6\frac{1}{4}$ figures. All the early samples were also treated so as to give the ammonia volatile with steam and 10 per cent. potash and the nitrogen insoluble in various "protein precipitants." In the end it was found that the N precipitated by basic lead acetate and the N volatile as NH_3 by steam and potash almost exactly added up to the total N and that other N precipitants were uncertain. Further the amount of "non-albuminoid" nitrogen as deduced from such estimations was too small in amount to have much

practical value. Normal lead acetate gave concordant results but left a considerable fraction unaccounted for. Copper hydrate gave very discordant results. The $(N = 6\frac{1}{4})$ precipitated by basic lead acetate added to the $(N = 6\frac{1}{4})$ volatile with steam and potassium hydroxide gave a result on the average of 70 tests of .005 per cent. above the total $(N = 6\frac{1}{4})$. The average difference between the sum of those two parts and the whole, neglecting signs, was .181 per cent. Of the 70 samples thus fully analysed the average "albuminoids" was 3.26 per cent. and the average "amides" was .29 per cent. or only 8.9 per cent. of the total.

I. EFFECT OF MANURE ON THE COMPOSITION OF OAT STRAW.

The compilation of averages of large numbers of trials, such as these, presents many difficulties. Classification is often rather difficult and some observations must be rejected as not capable of classification. By dividing the results of the analysis of oat straw into three groups we find the following figures. Unless otherwise expressed the results have been calculated from 1919, 1920 and 1921 crops.

A. 35 samples of oat straw grown with very much organic nitrogen such as ploughed in leas, apparently rich, omitting doubtful "clover takes" but including land with heavy dressings of dung.

Laevulose	1.65 %	$\pm .11$
Total sugar	2.51	$\pm .21$
Albuminoids	3.84	$\pm .13$

B. 29 samples of oat straw top dressed with sulphate of ammonia.

Laevulose	1.62 %	$\pm .21$
Total sugar	3.28	$\pm .24$
Albuminoids	2.54	$\pm .12$

C. 21 samples of oat straw grown with little if any nitrogenous manure in any form.

Laevulose	1.65 %	$\pm .28$
Total sugar	3.47	$\pm .45$
Albuminoids	2.57	$\pm .16$

The combinations of these results which give significant differences are: Much organic nitrogen gives an oat straw richer in albuminoids than that given by little or no nitrogen, to the extent of $1.27 \% \pm .22$, as judged by 56 tests. Much organic nitrogen gives an oat straw richer in albuminoids than that given by sulphate of ammonia top dressings, to the extent of $1.30 \% \pm .21$, as judged by 64 tests. Organic nitrogen manures give oat straw richer in albuminoids than that given by all other manures, to the extent of $1.28 \% \pm .17$, as judged by 85 tests.

Other probable results are: Organic nitrogen manures depress the amount of laevulose in oat straw to the average extent of $.59 \% \pm .21$.

Sulphate of ammonia is better than organic nitrogen for sugar production but only to a small extent.

II. EFFECT OF DISTRICTS.

It was quite impossible to do more than select a few farms to represent large areas. The conclusions arrived at can by no means be supposed to refer to the whole of the district alluded to. In some cases personal knowledge permitted the farms to be well scattered, so that County Durham is fairly well represented, but the district called Scotland is merely an average of a few results from Aberdeen and Edinburgh and is therefore merely a name for places well to the north of Northumberland. Similarly Yorkshire is represented almost entirely by Garforth, only a few other places in the county being among the list of farms from which samples were obtained. The southern counties district is more widespread, as it includes Essex, Herts, Bucks, Hants, Wilts, Derby and Notts, and may fairly represent "the South" from a north countryman's point of view.

In spite of these drawbacks in the classification, a useful comparison may be made with the following results.

Albuminoids in Oat Straw in Different Districts.

Moving from North to South:

Scotland with 20 samples gives $3.23\% \pm .10$.

Northumberland and Durham with 26 samples gives $3.15\% \pm .14$.

Cumberland and Westmorland with 15 samples gives $4.42\% \pm .15$.

Yorkshire with 27 samples gives $3.09\% \pm .08$.

Southern Counties with 34 samples gives $2.74\% \pm .09$.

The outstanding result is the much higher amount of albuminoids in Cumberland and Westmorland. Oats are there a very important crop as they receive much more manure than is customary in other parts. They frequently follow old leas and often receive much direct application of dung. Stock are moreover the central feature of the system of farming and much care is taken of the beasts. Owing to the damper climate it is possible that the roots of the oats go on absorbing nitrogen to a late stage and hence keep on accumulating nitrogen; but the average figures for non-albuminoid nitrogen are not especially high, so that some other explanation must be looked for. The superiority of the oat straw on the farms tested in Cumberland and Westmorland can hardly be attributed to any other cause than an increased amount of available nitrogen in the soil.

If we put Cumberland and Westmorland aside and compare the other districts there is at once the striking result that albuminoids increase directly with latitude. The difference between the Scottish figure, and those from the southern counties is marked and is quite in accordance with the popular impression that straw can be fed in Scotland in a way in which it cannot be fed in the south of England. At Cockle Park one experiment with different seed dates showed that the total nitrogen in the crop per acre was not very different; with autumn sown oats, the large crop of grain took nearly all the nitrogen, but the spring sown oats gave only half the grain yield and left a straw very rich in albuminoids. It follows that in Scotland with its shorter growing season the grain will not be able to exhaust the straw to the same extent as it would in England and that therefore Scottish oat straw will on the average contain more albuminoids than English oat straw. The conclusion is in close accord with the results based on the statistics.

A partial answer is here given to the well-known question: why can cattle be fattened on straw and roots in Scotland and not in the south of England? It is due to the superiority of north country straw in albuminoids. Quite possibly along with the albuminoids may go those little understood food accessory substances already alluded to. Swedes and turnips are very poor in albuminoids and the superiority of northern straw in this respect may be the determining factor. At Cockle Park, in feeding trials on hay, the determining factor is often the percentage of albuminoids. North country hay is poor in albuminoids whereas north country oat straw is relatively rich, or one might say south country hay is relatively rich and south country straw relatively poor. These two facts together go a long way to explain the respective practices in feeding cattle. The variation in the albuminoids in oat straw grown in different districts may possibly be partly due to rainfall. In Scotland, Northumberland, Durham and Yorkshire the average rainfall at the places where the oats were grown was about 30 inches, but the Cumberland and Westmorland areas have an average rainfall about 45 inches and the southern counties area about 27 inches. Among other causes of high proportions of albuminoids may be placed a good supply of water. Oats that are cut green will often be cut green because the season is wet with the result that the straw contains more albuminoids. Hence the cattle relish the straw and the farmer says that the straw is "sweet" but it is rich in albuminoids and not particularly rich in sugar.

*The District in which Oats are grown and the amount of Sugar
in the Straw.*

Seasonal influences play such a great part in sugar production and content that 1920 and 1921 do not give the same relationships.

In 1920, Cumberland and Westmorland headed the list and Northumberland and Durham were only a little behind the four northern counties, giving total sugar $4.48\% \pm .29$ with the rest of Great Britain at $1.69\% \pm .12$. In 1921 however the southern counties gave $4.76\% \pm .34$ and the four northern counties $1.80\% \pm .15$ the average of all except the southern counties being $1.44\% \pm .10$. From these results it is clear that the southern counties made good use of the dry season of 1921.

As in former years samples of oat straw which contain much total sugar also contain much laevulose, so that the laevulose varies from 50 to 70 per cent. of the total sugar, but when the total sugar is low in amount laevulose is often absent. On the average of all results, poor and rich, the laevulose is a little under 50 per cent. of the total but even then it is the commonest sugar, since the remainder is divided between cane sugar and dextrose and perhaps traces of other sugars.

GENERAL CONCLUSIONS.

Fine weather during harvest appears essential for obtaining high percentages of sugar. Sugar gradually disappears from straw after harvest. When straw is very dry the loss is small, but if damp, sugar is readily lost. Under average practical conditions high sugar content is not common but, under careful management, oat straw six months old has given very high figures for sugar. Roughly it might be said that the more nitrogen a soil contains the more albuminoids there will be in the straw, but much will depend on the amount of grain produced.

The general impression obtained during the course of these investigations is that the reason why feeding oat straw and swedes is so successful in one district and not in another may be summed up in the phrase "good husbandry." When a farmer understands and is keen on cattle he obtains more dung, which gives him better quality straw and roots. Feeding these again skilfully to more beasts gives him still more and still richer dung until in the limit of practice he is able to feed beasts almost entirely on straw and roots because both are rich in albuminoids. The lowest figure for albuminoids is 1.12 per cent. and the highest 8.05 per cent.; a variation more than enough to explain any

difference in feeding value. Poor samples of hay are often below 8 per cent. albuminoids. The highest total sugar is 9.74 per cent. and the lowest 0.33 per cent. Old leas ploughed out and plenty of muck give high albuminoids; fine harvest weather gives sugars. It is good management that secures the benefits of these improvements in composition.

The Value of Research Grants.

Twenty-two years ago one of us started to answer the question "why can cattle be fattened on roots and straw in Scotland and not in England?" Limitation of time restricted the enquiry to lines which looked promising, with little direct result although the experience has in the end proved very valuable. Thanks to a Special Research Grant from the Ministry of Agriculture, a very moderate extra expenditure has enabled the enquiry to be prosecuted in a complete manner. Out of this came the idea that perhaps it was the albuminoids in the straw that was the foundation on which an answer could be given; further work has shown that that is undoubtedly the case, as far as the main part of the answer is concerned. Nevertheless the sugar is important in both swedes and straw and contributes a great deal to the feeding value, but probably it is the albuminoids in the straw that make the chief difference between the practice of north and south.

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NOTE ON THE MECHANICAL ANALYSIS OF HUMUS SOILS.

BY GILBERT WOODING ROBINSON, M.A.

*Adviser in Agricultural Chemistry, University College
of North Wales, Bangor.*

It is generally recognised that the mechanical analysis of soils containing large quantities of organic matter presents considerable difficulties and that in the case of peaty soils mechanical analysis can have little significance. Apart from the masking effect of organic matter on soil properties which will naturally vitiate any correlations with mechanical composition, the actual dispersion of humus soils is difficult owing to the cementing action of humified organic matter, whereby soil particles are aggregated together into compound structures which resist ordinary methods of dispersion. Various methods have been suggested for the destruction of organic matter as a preliminary to mechanical analysis. Atterberg¹ recommends the use of alkaline sodium hypobromite solution. In the case of diatomaceous soils, however, oxidation of the organic matter with hot nitric acid (*d.* 1.14) is recommended. For soils free from calcium carbonate the use of hydrochloric acid (*d.* 1.12) is suggested.

In view of the reactive character of some of the finer soil constituents these and similar methods would appear to be open to objection. Any acid treatment is certain to result in the partial solution of clay and finely divided minerals, whilst alkaline treatment results in the attack of silica or colloidal silicic acid.

The soils of North Wales are generally high in organic matter and the writer has long suspected that the figures for clay obtained in the ordinary mechanical analysis might be too low and that a certain amount of clay was reckoned with the other fractions. Two circumstances favoured this view. In the first place, the majority of the soils of this area are derived from rock material which might be expected to furnish rather heavy soils. Further the analyses of the hydrochloric acid extracts for such soils show high figures for silica, iron oxide and aluminium oxide such as might be expected from clay soils.

¹ *Int. Mitt. Bodenkunde*, 1912, 2, 312-342.

288 *Note on the Mechanical Analysis of Humus Soils*

Attempts were therefore made to effect the destruction of organic matter without attacking clay or fine mineral particles. This requires of course a neutral reagent. The first oxidising agent tried was ammonium persulphate. Considerable oxidation of the organic matter can be effected by the use of this reagent in aqueous solution but the sulphuric acid liberated dissolves mineral material and it is necessary to maintain neutrality by repeated additions of alkali. There is the further disadvantage of the large quantities of ammonium sulphate introduced which has to be removed before the soil can be subjected to mechanical analysis. With this somewhat inconvenient method, however, it was found that considerably higher figures were obtained for the clay fraction than by the ordinary method. Some results obtained on a typical Welsh soil using different methods of dispersion may now be given.

Preliminary treatment	Clay %
Ordinary method	6.9
One hour digestion with HCl (d. 1.12)	13.5
Digestion with NaBrO (Atterberg)	10.5*
Ammonium persulphate oxidation (10 : 20), No neutralisation	13.04†
Ammonium persulphate oxidation (5 : 20), Maintained approx. neutral	16.0

* 7.58 % of material dissolved.

† 6.48 % of material dissolved.

Microscopical examination of the fractions obtained showed striking differences. The fine silt and silt obtained by the ordinary method contained considerable quantities of amorphous material. The same fractions obtained after preliminary oxidation were seen to consist entirely of crystalline mineral matter. Similar fractions are obtained from raw clays and soils poor in organic matter.

The use of ammonium persulphate proved inconvenient in practice and hydrogen peroxide was therefore tried as an oxidising agent. After some preliminary trials it was found that the most convenient method of oxidising was as follows. Ten grams of soil are weighed into a beaker of 600-700 c.c. capacity. Fifty c.c. of hydrogen peroxide (20 vols.) are added and the beaker placed on a boiling water bath. A vigorous reaction soon takes place with considerable frothing owing to the evolution of oxygen. The contents of the beaker are stirred from time to time. After about 30 minutes the reaction dies down and a further 25 c.c. of peroxide are added, the froth adhering to the sides of the beaker being washed down with a small volume of water. After a further 15-20 minutes' heating the reaction is generally complete and frothing

ceases. In the case of soils with large proportions of organic matter, more peroxide will be needed. The beaker is then removed from the water bath and, after adding about 100 c.c. of water, boiled for about 15 minutes.

A considerable oxidation of organic matter has now taken place and the oxidised material acquires the yellowish or light brown colour of a non-humous subsoil. The contents of the beaker remain approximately neutral so that no solution of mineral matter may be apprehended. In order to form some idea of the effect of this oxidation on the soil organic matter two soils were oxidised. After filtration and washing the residual material was dried and the loss on ignition determined. The filtrate and washings were evaporated to dryness and the amount of soluble organic matter determined. A separate experiment was also carried out in which the gaseous products were collected in normal sodium hydroxide and the amount of carbon dioxide estimated by double titration. The amount of organic matter completely oxidised to carbon dioxide was found by multiplying the weight of carbon found as carbon dioxide by 2, assuming as a first approximation that the organic matter contained 50 per cent. of carbon. The results obtained were as follows:

Soil	Loss on ignition	Loss on ignition after oxidation	Oxidised to CO_2	Soluble org. matter
C 26	25.4	8.1	5.5	10.8
4 T	10.1	4.0	1.9	3.7

It will be seen that the original organic matter is approximately accounted for by the unoxidised matter and the products of oxidation.

A number of soils thus oxidised were submitted to mechanical analysis. The result of the treatment is to break down the compound particles very thoroughly and the separation of the fine gravel and coarse sand by means of the 100 mesh sieve is very easily effected with very little trituration. Generally speaking the sedimentations were carried out twice without the addition of ammonia and then as in the ordinary method. It may be added that the soluble products of oxidation would appear to be deflocculating in their action and quite considerable quantities of clay can be obtained without the use of ammonia. The following table shows the results obtained for a number of soils by the ordinary method including the usual preliminary treatment with HCl, and also by the hydrogen peroxide method, in which this acid treatment is omitted.

290 *Note on the Mechanical Analysis of Humus Soils*

Soil	Ab. 1 B		Ab. B S		F 2 A		F 2 B		C 26 A		C 36 A	
	Ord.	H ₂ O ₂	Ord.	H ₂ O ₂	Ord.	H ₂ O ₂	Ord.	H ₂ O ₂	Ord.	H ₂ O ₂	Ord.	H ₂ O ₂
Fine gravel	3.8	3.3	3.0	2.8	—	—	—	.1	3.8	4.0	7.8	10.3
Coarse sand	29.5	29.5	33.8	33.1	8.1	8.3	4.6	6.5	14.8	5.2	12.3	7.8
Fine sand	18.3	15.5	19.0	13.8	22.1	20.8	20.9	20.7	15.2	8.1	20.5	15.5
Silt	28.6	23.2	26.0	19.5	32.9	25.1	34.0	27.4	23.2	13.7	15.6	14.0
Fine silt	8.2	17.9	6.3	17.6	20.1	28.3	28.7	32.0	34.6	26.8	23.4	29.7
Clay									3.8	13.2	5.6	9.5
Moisture	2.3		1.3		3.5		3.2		3.3		2.6	
Org. matter	10.0		9.0		10.9		5.1		25.4		10.3	
Totals	100.7	101.7	98.4	97.1	97.6	97.2	96.8	95.6	101.1	99.7	98.1	99.7

Soil	G.A.C. 2		G.A.C. 3		G.A.C. 5		G.A.C. 11		G.A.C. 12	
	Ord.	H ₂ O ₂	Ord.	H ₂ O ₂	Ord.	H ₂ O ₂	Ord.	H ₂ O ₂	Ord.	H ₂ O ₂
Fine gravel	6.6	1.4	4.0	3.2	5.2	4.6	.9	.8	3.7	4.1
Coarse sand	25.7	27.9	20.5	23.0	22.5	24.9	23.6	25.3	11.8	12.2
Fine sand	27.1	20.4	29.7	21.0	27.0	18.7	26.8	21.4	32.8	18.6
Silt	10.8	10.5	14.8	10.5	15.7	12.5	16.0	12.0	20.0	17.5
Fine silt	10.7	17.7	10.1	17.2	12.5	19.0	13.2	15.7	13.1	19.0
Clay	3.8	6.8	3.8	8.0	4.2	7.5	3.5	9.3	3.3	13.0
Moisture	5.2		4.2		2.1		3.8		2.6	
Org. matter	8.1		10.9		8.7		9.6		11.0	
Totals	98.0	98.0	98.0	98.0	97.9	98.0	97.4	97.9	98.3	97.0

It will be noticed that in every case there is an increase in the amount of clay obtained. In other words the effect of the oxidation has been to increase the degree of dispersion of the soil. That this effect was not an apparent increase in the clay owing to higher viscosity of the aqueous solution of the oxidation products was shown by a viscosity determination on a filtrate obtained from an oxidation. The viscosity was sensibly the same as for pure water and it may be assumed that the difference is due to an actual dispersion of complex particles which are not broken up in the ordinary method of dispersion. The hydrogen-peroxide dispersion has of course been effected without preliminary acid treatment, in the case of the soils used. Possibly it might be desirable to include the acid treatment in the case of soils containing much carbonate. It is worthy of remark that the clay liquor obtained by the peroxide method is strikingly different from that obtained by the ordinary method. On agitation, it shows a satin-like effect owing to the reflection of light from minute crystalline particles. This effect is not shown by the clay liquor from soils in the ordinary method. Similar results are obtained with fine silt suspensions. It is also noticed that on flocculation of the clay liquor a smaller volume of flocculated material is obtained than in the ordinary method.

No attempt has been made to follow the chemical changes involved in the oxidation of soil organic matter by hydrogen peroxide. It may be mentioned that the soluble compounds formed appear to form a very suitable medium for the development of moulds and fungi as, when exposed to air, they quickly become covered with a scum on which growths appear. The products of oxidation would be well worthy of chemical examination.

The results obtained particularly in the case of Welsh soils indicate that the figures obtained for clay by the ordinary method may be misleading and that there are considerable quantities of 'clay' which are wrongly grouped with the coarser fractions. In other words the ordinary method fails to secure the prime particle structure. This defect in the standard method may operate to some extent even in soils with smaller amounts of organic matter, for in every case larger figures were obtained for the clay after peroxide treatment.

Treatment with peroxide offers a convenient means of removing organic matter from soil without altering the mineral portion. In these experiments it was not found possible to remove all the organic matter. Complete removal might be effected by successive treatment with peroxide. It would appear, however, that the humified organic matter is completely oxidised or rendered soluble for the dark colour is removed even from peat soils and the oxidised soil has the appearance of a raw subsoil. The unoxidised material in such cases appears to consist entirely of structural organic matter, a circumstance which suggests that only humified matter is attacked.

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A BACTERIAL DISEASE OF TURNIP (*BRASSICA NAPUS*).

BY S. G. JONES, M.Sc.

Lecturer in Botany, University College of Wales, Aberystwyth.

(With Plate III.)

OF recent years a disease of root-crops known to farmers in North Wales is one in which the heart or core of the root is converted into a soft putrid mass but which leaves the rind and mature foliage intact. The disease is prone to appear on land treated with lime as a preventive against the roots being attacked by *Plasmiodiophora brassicae* or when the land has received a heavy dressing of nitrogenous fertilizers. It is common knowledge that nitrogenous manures have a tendency to force the crop and so produce watery, sappy roots which easily fall prey to disease. Recently it was brought to the writer's notice by one of the County Organizers for Agriculture in Wales that some of the farmers in his area were strongly disinclined to lime the land for root-crops because this treatment was favourable to the appearance of "soft-rot." There is probably some modicum of truth in this contention, for as will be seen below, the writer in the investigation of the present disease found that the organism isolated refused to grow on any media which were not neutral or alkaline. The writer observed and investigated this disease on a crop of white turnips grown on the farm of the University College of North Wales and the land here had received a dressing of nitrate of soda. A casual glance at the crop did not show anything unusual, in fact judging from the amount of green foliage the crop looked very healthy. Closer examination however showed that the very young leaves at the centre of the crown had been destroyed thus forming a tiny wound into which one could push a probe without obstruction to the depth of some three or four inches. The fully expanded leaves very effectively concealed the wound and the disease seemed evidently confined to the internal tissues leaving the foliage and rind quite firm even up to time of harvesting the crop. Indeed the extent of damage was only fully revealed at the time of lifting when the harvester's knife

lopped off the foliage only to discover that the roots were bad. A diseased root cut in vertical section showed a flask-shaped, soft, putrid core surrounded by a brown zone abutting on the healthy tissue (Fig. 1). The disease never made further progress into the rind nor into the lower part of the root and this would therefore account for the healthy turgescence appearance of the foliage since the vascular tissue in the root and rind would still be functional. Such were the external features of most of the diseased plants but in addition numerous cases were found where the entire apical bud had been destroyed thus forming a large wound only however to be concealed by no fewer than three, often five secondary crowns all bearing healthy luxuriant foliage (Fig. 2). There were also found numerous diseased roots with all foliage intact, but bearing deep cracks in the rind above ground; these were probably examples of "burst" roots a condition frequently found in sappy roots exposed to sudden weather changes. In all these types however the internal appearance of the diseased roots was precisely the same—the soft-rot was always confined to the core of the root and in addition there was always present the brown-coloured zone at the boundary of the diseased area. A white-rot of turnips similar to the one now described has been attributed by Potter to an organism *Pseudomonas destructans* (Potter). He states that plants attacked by this parasite "can be recognised by the drooping yellowish leaves, the older leaves being the first to show any indications of disease; they gradually flag and droop to the ground, at the same time becoming yellow and shrivelled in appearance. The leaves next in age gradually exhibit the same signs of premature decay and this proceeds until finally the young leaves at the growing point succumb; the entire rosette of leaves perishes, and the whole root becomes a soft, putrid mass, which eventually collapses etc." In the present disease, however, the external appearances of the diseased plants showed features very different from those accompanying the one described by Potter and these were deemed sufficiently striking to warrant further investigation.

It has been a matter of extreme difficulty to detect the initial mode of attack of this disease on the plants in the field. The writer has not been able to investigate this important point and the evidence gathered from the farmers is most conflicting. One noticed that the turnip plants were to all appearances dead, with the leaves flat on the ground as if the cattle had been lying on them but that on a subsequent visit he had found the crop to all appearances fully recovered only to find however at the time of harvesting that the roots were bad. Another said that

he had also seen⁵ the leaves drooping but that the seeming recovery of the foliage was due to new growth from secondary crowns. At the College farm the writer found intermixed in the drills along with diseased roots numerous healthy-looking plants possessing brown, dry, empty cavities with no sign of the pasty mass ever having been present; such plants invariably harboured slugs or their ova. These plants had, like the types of diseased roots already described, either the young foliage at the growing point destroyed or the foliage of the apical bud had entirely disappeared with the wound thus formed completely healed and surrounded by luxuriant foliage from secondary crowns.

THE NATURE OF THE DISEASE.

With a view to determine the nature of the pasty mass in the core, small quantities of it, taken from several affected plants were placed in a little sterile water in a watch-glass. Microscopic examination of the turbid water showed isolated cells and cell-clusters floating in the liquid. There were no traces of protozoa such as are described by Priestley or of fungal hyphae. A small quantity of the liquid taken up on a sterile platinum loop and smeared on a cover-glass showed when stained a dense mass of bacteria. Cultures were then prepared for isolating the organisms.

METHODS.

The sterilization of apparatus and media was effected in the usual way. In the first instance nutrient gelatine acidified to 1 per cent. normal hydrochloric acid was employed but this medium was found unsuitable; then turnip-juice gelatine of natural acidity and neutralized was also used. Growth on these media, however, proved so inferior to that produced on beef-extract-peptone-gelatine neutralized with sodium hydrate that this was exclusively employed in the preparation of pure cultures. Precautions were taken to ensure its constant composition and uniformity of treatment in sterilization.

Diseased plants from which cultures were to be taken were thoroughly washed free from soil and placed for 15 minutes in a weak solution of corrosive sublimate. Without removing the chloride the roots were cut open with a sterile knife from root-tip to crown, the root being inverted in order as far as possible to avoid contamination with foliage epiphytes. Petri-dish cultures were then carried out in the usual way and the several colonies transferred to agar-slants.

The next step was to prepare sterile blocks of turnip for inoculation from the agar-streak cultures. By means of a platinum hook inoculations

were effected from the agar-streaks into small excavations made at the centre of the blocks. After an incubation period of 15 to 24 hours at 20° C. most of the blocks showed a whitish-grey transparency around the inoculated part. In the more juicy blocks, the affected area, in 24 hours, would be about the size of a sixpence. After further incubation for 24 hours they had become completely diseased; during the next two or three days they assumed a yellowish hue and after a week a brown colour. The organisms causing the rot in the blocks had been derived from the agar-streaks which had been taken from round, whitish-grey liquefying colonies. Repeated cultures from field material showed that the colonies effective on the turnip blocks were of this type. Poured plates taken from successfully inoculated blocks showed the colonies in crowded growth to be small, those situated below the surface of the gelatine being smaller. On a thinly sown plate the colonies were large, circular, with much liquefaction in the centre. Magnified under the low power of the microscope they showed a finely, closely fibrillated margin; within the margin was a narrow finely-granular zone, then a narrower denser granular band forming a very faint concentric circle within which was a wide zone gradually diminishing in a granular density towards the centre. The saucer-like depression contained massed bacterial debris floating in a thinly granular liquid. Buried colonies were small, round, fibrillated at the margin and homogeneously granular.

THE ORGANISM AND ITS FLAGELLATION.

After repeated cultures had been made in nutrient gelatine from the diseased blocks there remained no doubt that a pure culture had been obtained. Cover-slip preparations of the organisms stained with carbol-fuchsin showed them to be rod-like in form and of varying length. Single specimens were short with rounded ends and pairs were frequently seen. Hanging-drop cultures in bouillon from several young agar-slants showed the organisms to be actively motile. After repeated attempts to stain the flagella, it was found effective to transfer the organism from agar-streaks of not more than 12 hours' growth into a number of small petri-capsules half-filled with sterile, filtered water kept at the same temperature as that in which the agar-cultures were incubated. The cover-slips were placed in series of six in a large petri-dish bearing the corresponding number of the culture. A sterile platinum loop was then dipped into the small capsule and the drop quickly deposited on the cover-slip, six preparations being taken from the same capsule. These were air-dried

by immediately placing the petri-dishes in an incubator at 60°. Loedler's method of staining was first employed without success, but with this method, the organisms showed uniform staining, except at one pole which was somewhat hyaline. When however the mordant was made slightly alkaline with caustic-soda ($\frac{1}{2}$ c.c. of 1 per cent. alkali per 10 c.c. of tannin) most of the slides after staining showed the presence of a single long polar flagellum. Van Ermengen's stain, prepared according to the usual formula, also showed the presence of the cilium. No variation in the number or situation of the flagella was seen.

INFECTION EXPERIMENTS.

The next step was to establish the disease in healthy plants. It was decided to carry out infection of healthy plants by employing cultures from inoculated blocks. Preparations were accordingly made for inoculating a series of blocks from young cultures taken from the agar-streak tubes that had last been employed in the flagella staining. The latter process had entailed repeated failures and fresh attempts always involved the use of fresh subcultures. It was reckoned that the tubes now employed for inoculating the turnip blocks contained cultures which had passed through some 20 generations over the medium. After the usual period of incubation, the inoculated blocks showed little or no signs of disease. Some of the blocks showed a slight browning around the inoculated part and made no further advance; others did not appear to have taken the disease at all. It seemed that the organism had either lost its virulence in its repeated passages through the medium, or that the turnip, through prolonged cultivation had passed over into a state of resistance to attack. It was therefore decided to submit sterile blocks of turnip to special treatment on the lines followed by Laurent for rendering resistant tubers sensitive to bacterial attack, by immersing them in alkaline solutions. Accordingly the blocks were first soaked for an hour, some in 0.25 per cent., some in 0.5 per cent. and others in 1 per cent. caustic-soda solutions. They were then transferred to sterile test-tubes and inoculated from the same agar-streak cultures that had been employed on the supposed resistant blocks. After prolonged incubation the parasite made no more progress on the blocks thus treated than on the control ones which had only been soaked in water previous to inoculation. It was therefore concluded that the organism had lost virulence by so many passages through the artificial medium. When, however, another series of turnip blocks was inoculated from an agar-

culture derived directly from diseased material the usual signs of attack and rotting of the blocks took place.

As above mentioned the farmers who had witnessed the attack in the fields said that the disease had been preceded by a sudden collapse of the foliage. This reported phenomenon at once suggested infection of the leaves by way of the stomata or water-pores. Experiments were carried out on a number of plants in the following way. Uninjured leaves (attached to the plant) of varying ages were plunged into water in a series of petri-dishes to which had been added pure cultures of the organism from bouillon. The leaves were left immersed for an hour. In some plants they were first sterilized with weak mercuric chloride which afterwards was removed by repeated plunging into sterile water; in others, no surface sterilization was employed. The leaves of the control plants were also treated in the same way. These experiments failed to show any infection of the foliage. The writer is strongly of opinion that infection is preceded by mechanical injury through some such agency as leaf-cutting insects or slugs and the very earliest signs of disease in the very numerous cases seen in the field were the softening and water-soaked appearance of the young foliage. Further inoculation experiments were carried out by first moistening the young foliage at the growing point and then depositing a pure culture from bouillon. The root was then covered over with a bell-jar plugged at the top with cotton-wool. These experiments invariably failed despite the greatest care being taken to keep the inoculated part moist. Accordingly the tender foliage at the growing point was pinched off with a sterile forceps and into the wound thus made a pure culture of the organism was deposited, the wound being covered over with a piece of sterilized cotton-wool, and the whole plant again covered over as before. This method of inoculation was always successful but the progress of the disease varied somewhat in different plants. The extent of attack was determined from time to time by probing the cavity with a platinum wire. When a depth of some four inches had been reached the plants were cut open in vertical section. Those in which the pulp was watery showed the characteristic whitish-grey mass accompanied by the marginal brown discoloration. Other roots of a drier spongy texture showed a diseased core of a uniform brown colour. This difference of colour in the diseased parts suggested an idea that it might bear some relation to the water content of the cells and intercellular system or in other words to the extent of aeration of the tissues. During the earlier stages of the investigation it was noticed that when the inoculated turnip blocks in the test-

tubes were examined at intervals, some were always found to be more advanced in disease than others. Those which were spongy in appearance were brown or yellowish-brown in colour but the more succulent blocks were always whitish-grey. The difference of coloration in the diseased tissues may therefore be due to oxidation. Further proof of this was established by the appearance of the diseased blocks in the tubes after incubation periods of 24 hours, 48 hours, and 7 days. At first they showed a whitish-grey transparency around the inoculated part; later, they had become almost completely diseased but still whitish-grey, but after 7 days the blocks were considerably changed and distinctly brown in colour. The darkened colour, however, was only seen in those portions of the blocks exposed to the air in the tubes; the basal portions immersed in a little water were still light in colour. Still further evidence proving the discoloration to be due to oxidation was obtained by the following experiment. Two conical flasks with side tubes, plugged and sterilized in the autoclave were partially filled with sterile blocks about a cubic centimetre in size, and a little sterile water added. Two rubber stoppers which had previously been boiled in mercuric chloride and dipped in sterile water carried a bent tube, reaching to within half-an-inch of the turnip blocks. Into each a small fragment of diseased turnip was inserted, the stoppers quickly replaced and sealed with paraffin wax, sterile rubber connections furnished with pinch-cocks were slipped over the side and the bent tubes. The flasks were connected each to three similar flasks containing a mixture of pyrogallie acid and potash. A current of air was then drawn through the series of flasks for 15 minutes. The rubber connections of the turnip-flasks were then closed with the pinch-cocks and the flasks detached. From one turnip-flask the pinch-cocks were removed, and the tube-ends closed with sterile cotton-plugs. Both flasks were then incubated at 20° C. for a week. After 24 hours the blocks around the diseased fragment of turnip introduced showed the usual whitish-grey transparency. At the end of the week the contents of the cotton-plugged flask had become brown in colour, whereas the blocks in the sealed flask, all diseased, still remained whitish-grey in colour, and showed no sign whatever of browning, nor did they show any change of colour even after several weeks. Incidentally the experiment shows the organism to be facultative anaerobic. Further evidence of this property is given below.

As previously stated the discoloration in the field plants was confined to a distinct zone, between the healthy and diseased tissues. It is possible that the disintegration of the tissues following upon disease

would be attended by a collapsing of the cells, so that access of air through the diseased core would be prevented and thus hinder atmospheric oxidation. At the time of infection in the field, the plants would be actively growing and drawing upon the minerals in the soil. It is conceivable that one or more of these substances would have an oxidizing effect on the bacterial secretions in the diseased tissues, causing a discoloration at the margin of the diseased area. E. F. Smith states that Laurent, experimenting with different fertilizers on potatoes upon plots which had been treated with sodium nitrate and sulphate of ammonia produced tubers, which when inoculated with an organism (believed to be *B. coli*) gave a "black zone between the attacked and healthy tissues. As this stain was not noticed elsewhere, Laurent attributed it to the nitrogenous product formed by the bacteria at the expense of the tissues."

RELATION OF PARASITE TO HOST.

The relation of the organisms to the tissues of the host was first determined by examination of the diseased pulp of the field plant. A small quantity of the pulp mounted on a slide and gently pressed down with the cover-glass showed the cells to be completely isolated. The cell-walls were, however, intact and presented no appearance of being swollen. Numerous reticulated vascular strands were also seen with their walled prosenchymatous elements attached in places. The bacteria were generally seen outside the cells, but a few cells were so completely occupied by them that they appeared black amongst the contiguous unoccupied cells. Examination of the diseased pulp of inoculated plants showed exactly the same phenomena. Such densely occupied cells with the contiguous cells containing few or no bacteria were also seen in the microtomed sections of inoculated material.

Field material was fixed in Flemming's solution and in Carnoy's acetic-alcohol. Small pieces of the diseased roots were cut to include the healthy tissue, the brown zone, and the loose pulp. The slides made from the material fixed in the Flemming mixture showed almost the complete loss of the soft pulp in the prolonged process of washing. The alcohol-fixed material was therefore employed. The pieces selected for paraffin embedding included all the tissues from the periphery to the soft pulp. Transverse and longitudinal sections were cut on the microtome to a thickness of 4μ and mounted on slides thinly coated with white of egg. Considerable difficulty was experienced in the differential staining of the tissues and the bacteria respectively. The organisms

stained easily with the anilin dyes, particularly carbol-fuchsin, followed by differentiation in alcohol, showing the bacteria to advantage. Another method was to stain for 12 hours in Hoffmann-blue saturated with picric acid, and after washing in 50 per cent. alcohol, to immerse for three minutes in carbol-fuchsin. This showed the tissues blue and the bacteria red, but the staining was not always uniform and hence the method was unreliable. After repeated attempts with various dyes satisfactory results were obtained by the use of Heidenhain's iron-alum-haematoxylin followed by eosin in clove-oil. This showed the bacteria black and the cell-walls light red.

When the slides were examined under the low-power of the microscope, the bacteria were seen to be apparently exclusively confined to the intercellular system of the medulla, and the disintegration of the tissues to be brought about by the dissolution of the middle lamella. Some cells in the same region were seen to be densely filled with bacteria, relatively few or none being present in contiguous cells, thus repeating the features already observed in the examination of the diseased pulp. Some of the xylem elements also seemed to be occupied by bacteria. The microscopic examination of microtomed sections derived from artificially inoculated roots revealed an exactly similar invasion of the tissues to that seen in the field material. The bacteria abounded in the intercellular spaces of the inner tissues; occasional parenchyma cells were densely filled with bacteria, whereas the peripheral tissues were intact.

SEPARATION OF THE BACTERIA FROM THEIR PRODUCTS.

The separation of the by-products from the bacteria was then attempted. A two-litre flask containing a little water and sterilized by intermittent steaming was half filled with sterile blocks of turnip, a diseased block from a test-tube dropped in, and the flask quickly plugged. After four days' incubation at 20° the whole of the contents had been reduced to a pulpy putrid mass with a highly offensive odour. This was pressed through a piece of coarse linen and the turbid filtrate passed through filter-paper. The grey-coloured liquid was then divided into two portions—one portion was passed through a Berkefeld filter which had been sterilized for several hours in the hot-air oven and allowed to cool. The clear pale yellow filtrate was poured into a number of small, sterile test-tubes and corked with sterilized rubber bungs. To the other portion was added four times its bulk of 80 per cent. alcohol. After 24 hours a heavy flocculent precipitate had been formed. The super-

natant liquid was then siphoned out, the precipitate collected and washed with absolute alcohol. After careful drying in the incubator at 20° it was digested in 100 c.c. of sterile distilled water for three hours. The liquid was then passed through the Berkefeld filter. A clear pale yellow filtrate was again produced and poured into a number of sterile tubes plugged with cotton-wool and protected by rubber caps. The action of the two filtrates upon living turnips was then tried in the following way. Thin sections of turnips were placed in small petri-capsules, into some was poured the filtrate obtained from the pulp, into others the filtrate from the watery digest of the alcoholic precipitate. Other capsules contained sections in sterile water. Microscopic examination after 24 hours showed no differences between several preparations mounted and the control sections from the sterile water and examination again after several days showed no change in the appearance of the sections. Twelve tubes of the filtrates from both sources were employed upon the sterile turnip sections without any signs of loosening of the cells. The action of the filtrates upon a 1 per cent. starch solution was then tried, but the blue coloration after adding iodine still remained even after prolonged action. If an enzymic product capable of separating the parenchyma of the turnip was present in the diseased pulp, its power of action seemed to have been destroyed in the filter. A new series of experiments on similar lines was performed using a Muencke bacteria filter. This filtrate was again ineffective in loosening the tissues. The filtrates obtained from the watery digests of the alcoholic precipitates obtained from both bouillon and glucose-bouillon seemed to be quite inert in their action on the tissues. The use of the porcelain filters was then discontinued and the alternative method tried of treating the bacterial products with antiseptics. A solution of chloroform was employed. The culture treated was the turbid filtrate obtained directly from the putrid mass formed from diseased turnip. The coarser portions of the pulp were removed as before by pressing through a cloth and merely filtering the liquid through filter-paper. An equal volume of the chloroform solution was then added to the filtrate and the flask vigorously shaken at frequent intervals. To determine the sterility of the product inoculations into tubes of nutrient gelatine were taken and poured into petri-dishes. At the same time thin sterile sections of turnip were treated with the product. After 24 hours' immersion the tissues had become disintegrated and further the poured plates were sterile.

302 *A Bacterial Disease of Turnip* (*Brassica Napus*)

CHARACTERS OF THE ORGANISM.

HABIT. Causes a whitish-grey rot in turnip.

MORPHOLOGY. Bacteria 1.3μ to 3μ \times $.75\mu$ to $.9\mu$ with a single polar flagellum. On sugar-rich media it forms long, unequally segmented filaments.

RELATION TO OXYGEN. *Evidence for aerobism:* Large surface colonies and rapid growth on the surface of the media, buried colonies small; gelatine stab funnel-shaped; rapid decay of turnip in the cotton-plugged flasks and tubes. *Evidence for anaerobism:* Slight growth on recently steamed agar-slope tubes cultivated by Buchner's method for anaerobes; general turbidity in liquid media enclosed in Durham and Fermentation tubes; growth in gelatine stab after sealing mouth of track; decay of turnip blocks in a sealed flask.

GROWTH ON GELATINE MEDIA. Surface colonies large, round, whitish-grey, margin fibrillated. Liquefies gelatin; stab-culture funnel-shaped in 24 hours, spreading to wall of tube in 48 hours, liquefaction nearly complete. In the sealed track liquefaction not complete. Colonies on litmus-lactose and litmus-glucose-gelatine produce faint pink margin; on chalk-lactose and chalk-glucose-gelatine, hyaline ring; poor growth on acidified medium.

GROWTH ON AGAR MEDIA. Surface colonies round or slightly lobed, opalescent; sometimes spreading, filmy areas formed; deep colonies oval or spindle-shaped. Growth on agar-streak spreading and filmy. Good growth along agar-stab, spreading at surface. Colonies on litmus-lactose and litmus-glucose-agar produced pink margin; on chalk-lactose and chalk-glucose-agar hyaline border.

GROWTH IN BOUILLON. Renders bouillon turbid, forming a heavy sediment; no pellicle; maximum turbidity in neutral and feebly alkaline broth; neutral reaction to litmus; with 2 per cent. glucose, lactose, and cane-sugar turbidity in open and closed ends of Durham and Fermentation tubes, producing acid reaction without evolution of gas. In Pasteur's solution with cane-sugar turbidity only in open end of tubes.

GROWTH IN NITRATE SOLUTION. In Giltay and Aberson's culture fluid strong nitrite reaction after 24 hours with iodine-sulphuric-starch test, with formation of ammonia (Nessler's Test). Heavy sediment formed and film on surface of culture. Control tubes gave neither reaction.

GROWTH IN NITROGEN-FREE SOLUTION. No turbidity; after seven days, petri-cultures in gelatine inoculated from it produced very few colonies. Growth therefore feeble.

1. Produces a "white-rot" in turnips. In dry spongy tissues the colour is brown. Point of attack at the crown. Rind and foliage remain healthy.
2. Bacteria 1.3μ to $3\mu \times .75\mu$ to $.9\mu$ with a single polar flagellum. Long unequally-segmented filaments formed on sugar-medium. As far as could be ascertained from the staining of old cultures endospores are not formed.
3. Aerobic and facultative anaerobic.
4. Liquefies gelatin. Funnel-shaped depression in stab culture, gelatine almost completely liquefied.
5. Colonies on agar round or slightly lobed, white, opalescent. Buried colonies small, elliptical or spindle-shaped.
6. Parasitic on turnip, potato, carrot, radish, cabbage, but not on beetroot.
7. Turbidity in bouillon with sediment.
8. Presence of a cytase and diastase. Dissolution of cells along middle lamella. No swelling of cell-wall. Peptonising ferment produced which liquefies gelatin.
9. Acid produced in all sugar-bouillon. Presence of toxin undetermined. No direct evidence of oxalic acid. Ammonia produced in turnip, potato, carrot, and radish cultures.
10. Stains by Gram's method.
11. Organism lost vitality on the artificial media.
12. Reduces nitrates to nitrites.
1. Produces a "white-rot" in turnips. Point of attack at any wound on plant. Foliage withers and rind collapses.
2. Bacteria $3\mu \times .8\mu$ with a single polar flagellum. Long filaments not formed.
3. Aerobic.
4. Liquefies gelatin. Funnel-shaped tube in stab. Liquefaction extends only to depth of about $1\frac{1}{2}$ centimetres.
5. Forms white glazy growth on agar.
6. Parasitic on turnip, potato, carrot, but not on beetroot.
7. Renders Koch's bouillon and turnip cloudy and opaque.
8. Cytase produced with swelling of cell-walls; diastase with disappearance of starch; peptonising ferment liquefying gelatin.
9. Produced a toxin—oxalic acid. Residual product always acid.
10. Gram negative.
11. "Bacterium" can live for many generations as a saprophyte, without losing its virulence as a parasite."
12. ?
1. "In turnip-roots a water-soaked appearance of the flesh is not infrequent, but the brown stain also occurs." Bacteria enter by stomata or water pores, producing blackening of the vascular system.
2. Bacteria .7 to 3.0μ by 0.4 to 0.5μ with a single polar flagellum. Long filaments formed. Endospores not formed.
3. Aerobic.
4. Liquefies gelatin. Growth in stab cultures usually best near the surface. Only upper part liquefied.
5. Colonies round, pale-yellow, finely granular. Buried colonies small and elliptical.
6. Parasitic on several vegetables including turnip, potato, carrot, radish, cabbage.
7. Causes turbidity in bouillon.
8. Middle lamella dissolved. Destroys potato starch. Peptonising ferment produced.
9. "If any acids are produced the presence of air is required, and they are readily obscured by the production of ammonia."
10. ?
11. Loses in virulence on the media.
12. Doubtful. Smith states that it does not reduce nitrates, but says that Fischer proclaims it to be a nitro-bacterium.
1. Forms a brownish-black or whitish growth in turnips.
2. Bacteria 1μ to $3\mu \times .5\mu$ to $.6\mu$ with 7 to 13 peritrichous flagella.
3. Aerobic.
4. Liquefies gelatin. Forms a funnel-like growth in the tube along the puncture.
5. Forms white opalescent growth on agar.
6. Grows on living turnip, potato and carrot, but not on beet.
7. Renders bouillon turbid with sediment.
8. Produces eytase, diastase and a peptonising ferment.
9. Acid produced in all sugar-media and in bouillon, while ammonia was produced in potato, turnip, and carrot cultures.
10. Gram negative.
11. ?
12. ?

† Smith, *Bact. in rel. to Pl. Diseases*, 2, p. 303.

‡ Johnson and Adams, *Proc. of Royal Dublin Society*, 2, No. 1

304 *A Bacterial Disease of Turnip* (*Brassica Napus*)

PARASITISM. Produces a soft-rot in swede, potato, carrot, radish and cabbage. No growth on beetroot.

FERMENTS. Pectinase; diastatic and peptonising ferments produced.

REACTION OF BY-PRODUCTS. Diseased pulp neutral or feebly alkaline; bouillon neutral; acid produced in sugar media; production of ammonia in the vegetable media. CO_2 evolved from diseased pulp.

REACTION TO STAINS. Stains easily with various anilin dyes and by Gram's method. With Loeffler's flagella stain, one pole hyaline.

CONCLUSION.

While the organism under consideration has many characters in common with *Pseudomonas campestris* (Smith) and with *Bacillus Oleracea* (Harrison) it is clearly most nearly related to *Pseudomonas destructans* (Potter). The writer is of opinion that it is a varietal form of the latter. Of the differences mentioned in the table the most striking is the mode of attack of the disease while the root retains its shape, with the rind firm and the mature foliage healthy.

This investigation was carried out in the Laboratory of Agricultural Botany, University College, Bangor. It was taken up at the instance of Prof. J. Lloyd Williams (formerly Adviser at Bangor) who had observed this disease on farms in North and South Carnarvonshire, but his enquiries in the other Counties failed to bring to light any other instances of its occurrence, although many cases were observed of other rots of swede and turnip. To him the writer wishes to express great indebtedness for guidance and kindly advice and also to Professor R. G. White by whose courtesy he had access to the Laboratory and the College Farm.

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(b) On the parasitism of *Pseudomonas destructans*. *Proc. of Royal Society*, **70**.
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Fig. 1



Fig. 2

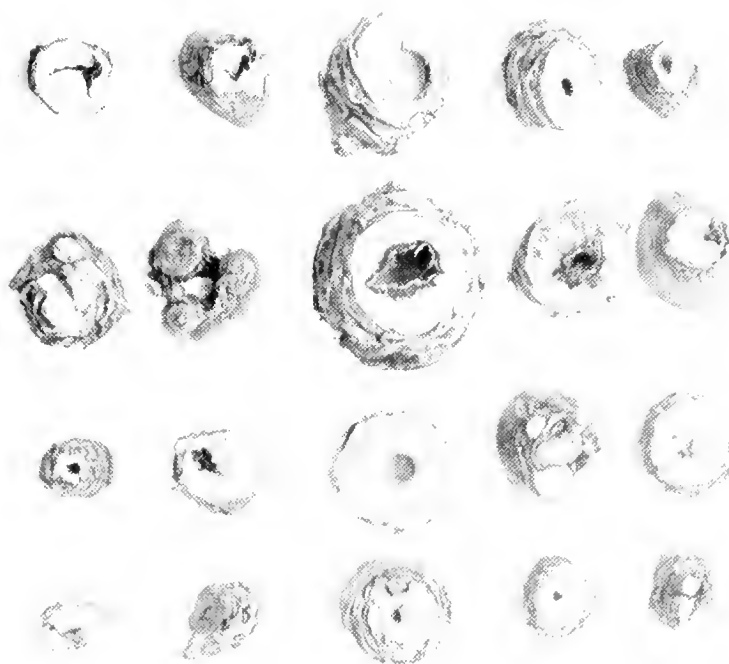


Fig. 3

- (5) PRIESTLEY and LECHMERE. A bacterial disease of Swedes. *Journ. Agric. Sci.* **3**.
- (6) SMITH, ERWIN F. *Bacteria in Relation to Plant Diseases* (Washington), **1**, **2** and **3**.
- (7) TAYLOR, T. H. Cabbage Top in Swedes. *University of Leeds and The Yorkshire Council for Agricultural Education*, No. 82.

EXPLANATION OF PLATE III.

- Fig. 1. Diseased turnip with the young foliage of the growing point absent. The plant had been left in the open and only removed at the time of winter ploughing. The rind had become hard, dry, and cracked. A small quantity of the shrunken pasty mass is shown.
- Fig. 2. Appearance of the crown of a plant with six secondary shoots. The aperture enclosed by them is surrounded also by leaf scars, which evidently belonged to the decayed apical bud.
- Fig. 3. Sections across the leaf-crowns of diseased plants.

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A NEW METHOD FOR THE MECHANICAL ANALYSIS OF SOILS AND OTHER DISPERSIONS.

BY GILBERT WOODING ROBINSON, M.A.

*Adviser in Agricultural Chemistry, University College
of North Wales, Bangor.*

INTRODUCTION.

ATTENTION has been given of recent years to the possibility of devising methods of mechanical analysis which shall express the mechanical composition of a soil or clay by a continuous curve. The standard methods of mechanical analysis, as for example that followed in England, can of course be used to obtain such curves, but with the comparatively small number of fractions separated, very little detail can be inserted. Any multiplication in the number of fractions must inevitably be attended by an increase of experimental errors, and the problem must therefore be attacked by other methods.

Odén has devised an elegant method whereby the mechanical analysis of a soil or clay can be derived from an experimental curve showing the rate of accumulation of sediment from a column of material in suspension¹. The weight of material is automatically registered from time to time and from the curve obtained a mass distribution curve is derived which gives a detailed representation of the composition of the material under examination. Wiegner² has applied the theoretical principles of the Odén method in a very simple apparatus consisting essentially of a U-tube system in which a column of sedimenting suspension is balanced against a column of pure water. As the material falls below the point at which the water tube joins the fall tube, the specific gravity of the suspension decreases, resulting in a corresponding fall in the water column. The height of the water column is read from time to time and

¹ *Int. Mitt. Bodenkunde*, 1915, **5**, 257-311; *Koll. Zeit.* 1916, **18**, 33-48; *Trans. Faraday Soc.* 1922, **17**, 327-348; Nefedof, *J. Exp. Landw.* 1902, **3**, 421-449, outlines a method similar in principle to that of Odén, but apparently purely empirical.

² *Landw. Versuchs Stat.* 1918, **91**, 41.

by appropriate calculation the experimental results can be thrown into the form of curves similar to those obtained by Odén's instrument. Both Odén's and Wiegner's methods have found applications in the study of dispersions¹.

A serious drawback to Odén's apparatus is its expense. While it is of the highest value for the critical investigation of comparatively small numbers of samples, it can hardly come into use for routine work as the resources of an ordinary provincial institution would scarcely be equal to the outlay involved in setting up more than one such instrument and only comparatively small numbers of soils could be dealt with. Wiegner's apparatus is cheaper and could by modifications² be made to give results of considerable accuracy. But here the time factor is serious. Using, as is necessary, a column about a metre long, several days would be required to obtain information as to the finer fractions which exert such an important effect on the properties of the soil.

In the present work the writer has attempted to devise a method capable of giving more detailed data than the ordinary sedimentation methods and which, though not giving continuous curves, avoids the drawbacks of the Odén and Wiegner methods and can be used for standard mechanical analysis with great saving of time.

Before discussing the new method of mechanical analysis a few remarks may be made on the expression of mechanical composition by means of curves. The simplest method of expressing the results of a mechanical analysis is to plot summation percentages³ against particle sizes. Such a method is however almost useless in the case of highly dispersed substances, because in order to show the complete range the most characteristic particle sizes are cramped together near the y -axis. A better distribution will be obtained by using the logarithms of particle sizes. The end of the curve corresponding to zero size is of course at $-\infty$ but in practice a very manageable type of curve will be obtained⁴.

Since the separation of particles of diameter smaller than $\cdot 2$ mm. is universally based on the principle of subsidence, whereby fractions are distinguished by their different settling velocities in water, there are good reasons for using logarithms of settling velocities instead of logarithms of particle sizes. In the ensuing treatment this method is used, the velocities being calculated in ordinary c.g.s. units. The same

¹ *Koll. Zeit.* 1920, **26**, 100–121; *ibid.* 1920, **26**, 121–138. *J. Landw.* 1921, **69**, 5–32.

² As for instance in the method of reading the height of the water column.

³ *i.e.* percentages of material of a given particle size or smaller.

⁴ Cf. Whittles, *J. Agric. Sci.* 1922, **12**, 166–181.

type of curve will be given and it will dispense with any assumption as to the size, shape and density of the settling particles.

In this connexion, the probable effect of the gel coating which has been postulated for the finer particles of a soil or clay on the sedimentation of such material is worthy of consideration. It has been suggested that such coatings are not to be considered as uniform and discrete but as concentric shells of increasing degree of hydration. As an approximate basis of calculation let us assume that the emulsoid coating has the same density as the water in which the particles are suspended. What will be the effect of this coating on the settling velocity? If a be the radius of the falling particle and d the thickness of the gel coating or shell, then the velocity of fall of the particle in the absence of any coating is given by

$$v_1 = \frac{2}{9} \cdot \frac{ga^2}{\eta} \cdot (\rho - 1),$$

using the usual notation and assuming the density of water = 1, and the velocity of the coated particle by

$$v_2 = \frac{2}{9} \cdot \frac{g(a+d)^2}{\eta} \cdot (\rho_1 - 1),$$

where ρ_1 is the mean density of the coated particle.

$$\text{Now } \rho_1 = \frac{\text{weight of particle + shell}}{\text{volume}}$$

$$= \frac{\frac{4}{3}\pi a^3 \rho + \frac{4}{3}\pi [(a+d)^3 - a^3]}{\frac{4}{3}\pi (a+d)^3} \\ = \frac{a^3 \rho + [(a+d)^3 - a^3]}{(a+d)^3}.$$

$$\text{Then } v_2 = \frac{2}{9} \cdot \frac{g(a+d)^2}{\eta} \cdot \left\{ \frac{a^3 \rho + [(a+d)^3 - a^3]}{(a+d)^3} - 1 \right\} \\ = \frac{2}{9} \cdot \frac{g}{\eta} \cdot \frac{a^3(\rho - 1)}{a+d}.$$

$$\therefore \frac{v_1}{v_2} = \frac{a+d}{a}.$$

$$\text{or } v_2 = v_1 \cdot \frac{a}{a+d}.$$

It will be seen that the presence of a coating of approximately the same density as the suspension medium will have a marked effect on the velocity of fall. The low velocities corresponding to the finer fractions

will thus be conditioned not simply by the actual particle sizes but by the magnitude of the gel coating. The writer hopes to return to this point in a later paper. In the meantime it is suggested that the left hand portion of the curve in the case of clay soils may relate to such coated particles rather than to particles bounded by a solid surface. By using $\log v$ instead of \log particle size, the necessity for a decision as to the significance of these small velocities is postponed.

It is of course obvious that the expression of the mechanical composition of a soil or clay by a curve of this type offers a way out of the appalling confusion created by the diversity of conventions used in different countries. Since practically all the methods in use are based on the principle of sedimentation, curves can easily be obtained if the settling velocities are known. Whether the principle is applicable to methods in which separation is effected by currents of water of varying velocity, as in the Hilgard method, the writer is unable to decide. As a first approximation, it would appear that the method is applicable.

We have assumed that the viscosity coefficient in the Stokes' equation is constant. With varying temperatures this is of course not the case. This difficulty can however easily be solved if a standard temperature be adopted, say, 15°C . By putting the viscosity coefficient of water at that temperature equal to unity and calculating the viscosities at other temperatures¹ with reference to this, the correction can be applied. Thus if results obtained at varying temperature are to be compared, it will be necessary to use $\log (v \times \text{specific viscosity})$ instead of $\log v$.

THEORY OF NEW METHOD.

A fundamental assumption underlying all methods of mechanical analysis by sedimentation is that particles in a column settle independently of each other. That there are limits to this assumption is obvious. According to Odén this condition is fulfilled in suspensions of concentration not greater than 1 per cent.² Wiegner, on the other hand, brings evidence to show that concentrations of more than 5 per cent. can be used without serious inaccuracy, which may be due to the dangerous principle of compensating errors.

Let us assume a suspension of soil or other granular material to consist of a number of fractions, a , b , c , etc., each uniform in itself, having limiting velocities, v_1 , v_2 , v_3 , etc., respectively, and present in

¹ For the effect of temperature on the viscosity coefficient of water, see Hosking, *Phil. Mag.* 1907, **17**, 509; *ibid.* 1909, **18**, 260.

² *Int. Mitt. Bodenkunde*, 1915, **5**, 276.

310 *Mechanical Analysis of Soils and other Dispersions*

concentrations, A_1, A_2, A_3 , etc., respectively such that $\Sigma A = C^1$, the total concentration. Then if the fractions settle independently of each other, each fraction will behave as a separate column uniform in concentration from top to bottom and we may represent the state of affairs at the beginning of sedimentation as in the upper diagram of Fig. 1, the relative amount or partial concentration of each fraction being represented graphically by the thickness of its column on the diagram.

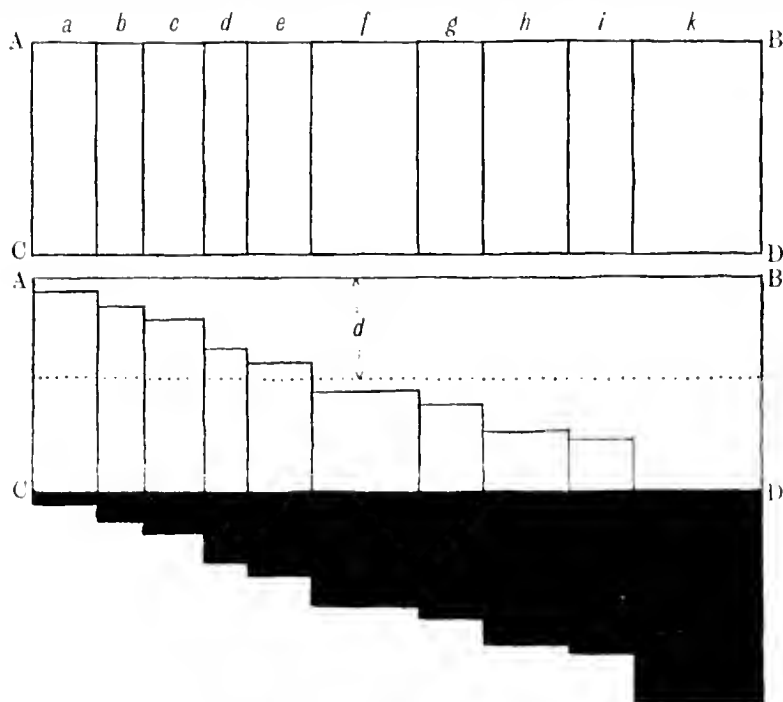


Fig. 1. Diagrammatic representation of sedimentation.

As sedimentation proceeds each column will fall bodily at its own appropriate velocity and the disposition after settling has proceeded for a certain time may be represented by the lower figure of the diagram. The black portion below the line CD will represent the amount of each fraction accumulated on the bottom of the sedimenting vessel, while the concentration of the suspension at any depth will be given by the total width of columns at that depth. Thus at depth d , the concentration will be equal to the sum of the partial concentrations of the

¹ Or $\Sigma A + \text{organic matter} = C$, in the case of ordinary soils.

fractions, a to e , having velocities less than d/t . The ratio of the concentration at depth d after time t to the total concentration at the beginning of the experiment will thus give the proportion of material having velocities less than d/t . By determining the concentration for different values of d/t the data are obtained for a summation curve showing the relation between percentage of material and log settling velocity.

EXPERIMENTAL.

The method used consists in allowing a soil suspension of known concentration to settle in a cylindrical vessel and withdrawing samples for appropriate values of depth/time. By suitable choice of depth and time the concentration and hence the percentage of particles corresponding to any desired velocity can be obtained. Generally speaking a litre measuring cylinder about 40 cm. in height and 6 cm. in diameter is used. There is of course no necessity to use a graduated vessel: any cylinder of uniform cross section and suitable dimensions may be used. Sampling of the suspension is carried out by means of a 20 c.c. pipette passed through a cork or shive and adjusted so that when the cork rests on the top of the cylinder the point of the pipette is at the desired depth below the surface of the liquid (see Fig. 2). The column having settled for the required time the pipette, previously adjusted for depth, is closed at the top with the finger, in order to avoid sampling the upper layers, and lowered very carefully till the cork rests on the top of the cylinder. The finger is then removed and 20 c.c. of the suspension withdrawn. Every precaution is of course taken to avoid shaking or mixing the layers of the suspension at the point of sampling. With a column of the dimensions mentioned the withdrawal of 20 c.c. causes a fall in level of about 7 mm. This probably represents the extreme error in sampling. It is assumed that the 20 c.c. of suspension withdrawn represents the concentration at the point of the pipette. Probably the liquid comes mainly from above, but to some extent from below this point. A separate experiment with a column which had settled for several weeks and which had formed clearly defined strata, showed that it was possible to pipette to within 2 to 3 mm. of a stratum without disturbance. It will be shown later that an error of a few millimetres in sampling involves a negligible error in the final result. Careful manipulation is of course necessary in this operation. The 20 c.c. of suspension

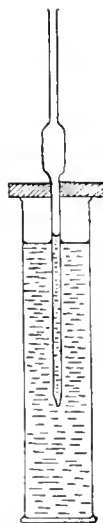


Fig. 2.
Method of
sampling.

is delivered into a flat porcelain dish which has been previously ignited and weighed. Dishes ordinarily used for the estimation of total solids in milk are convenient for the purpose. The sample is taken to dryness on the water bath and, if the estimation is to be made on unignited material, weighed after attaining constant weight. Ordinarily it is ignited in a muffle, an operation which only takes a few minutes at red heat, and weighed after cooling in a desiccator. From the weight of ignited material the concentration of the sample of suspension is calculated. By sampling in such a way that successively smaller values of depth/time are used, the same suspension may be shaken up and sampled over and over again. The partial concentration of any fraction at a given depth is unaltered until the top of the fraction column has sunk below that depth, as will be seen by reference to Fig. 1. The removal of a sample of suspension does not therefore affect the concentration of the suspension with respect to fractions of smaller velocities.

In an actual experiment a 2.5 per cent. suspension of a clay was prepared by shaking up 100 grams of powdered clay for 24 hours with 2 litres of water containing 100 c.c. of 1 per cent. sodium carbonate solution and making up finally to 1 litre. A litre cylinder was then filled to within 3-4 cm. of the top with the well mixed suspension and after again shaking for a minute, allowed to stand for six minutes. A 20 c.c. sample was then withdrawn at 36 cm. depth as described above. After drying and ignition, the weight of ignited material was found to be .376 gram. Subtracting .005 gram for the amount of sodium carbonate in 20 c.c., we have the nett weight of ignited material as .371 gram and the concentration of the suspension at the point sampled, 1.855 per cent. The original concentration being 2.5 per cent., we find that the concentration of the suspension at 36 cm. after six minutes is

$$\frac{1.855}{2.5} \times 100 = 74.2 \text{ per cent. of the original concentration.}$$

In other words 74.2 per cent. of the clay, reckoned as ignited material, has a settling velocity less than .4 cm./sec. Other determinations were made for successively smaller velocities and the results are set out in Table I. For the sake of brevity the weight of ignited material is given after subtraction of the .005 gram of sodium carbonate.

In Fig. 3 curves are shown for the clay of the experiment just described and for a few other typical clays and soils. The vertical dotted lines, *A*, *B*, and *C*, are the ordinates corresponding to clay, fine silt, and silt, respectively, according to their settling velocities in the English method. In order to bring the fine sand, coarse sand and fine gravel

Table I. *London Clay*. 2.5 per cent. suspension in .025 per cent. sodium carbonate solution. Temperature 12–16°.

Time secs.	Depth cms.	Velocity cm./sec.	$\log v$	Ignited material in 20 c.c. gms.	Concen- tration %	% of original concen- tration
360	36	.1000	1.0000	0.371	1.855	74.2
600	20	.0333	2.5227	0.358	1.790	71.6
600	6	.0100	2.0000	0.325	1.625	65.0
6000	20	.0033	3.5227	0.293	1.465	58.6
6000	6	.0010	3.0000	0.252	1.260	50.4
60000	20	.00033	4.5227	0.220	1.100	44.0
60000	6	.00010	4.0000	0.178	0.890	35.6
600000	20	.000033	5.5227	0.135	0.675	27.0
600000	6	.000010	5.0000	0.093	0.465	18.65

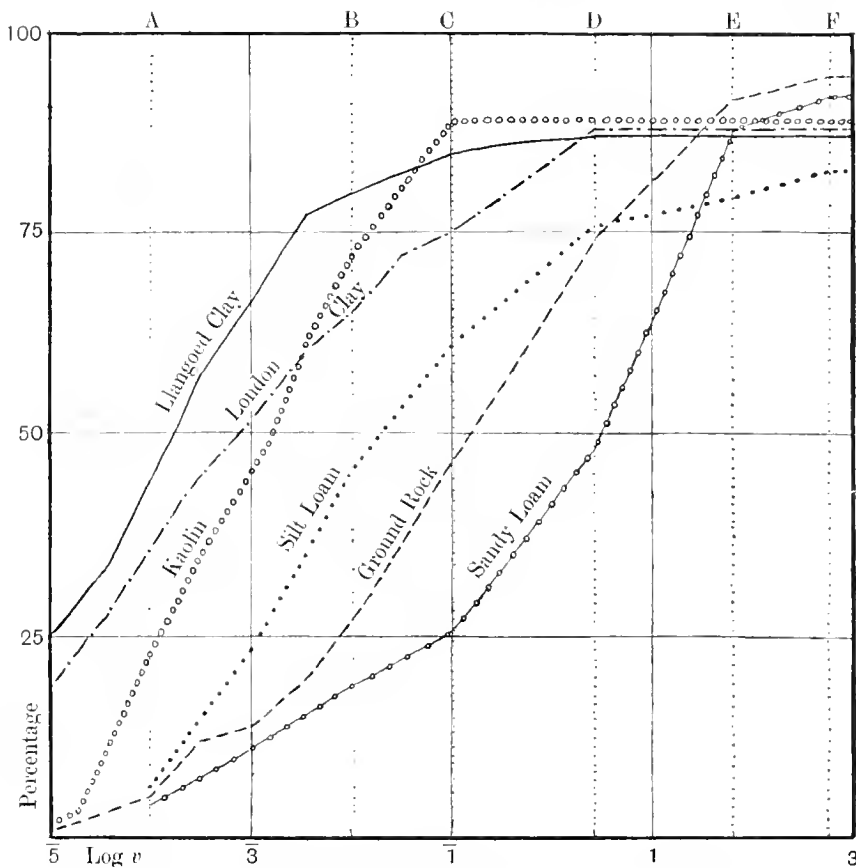


Fig. 3. Summation Curves showing Composition of Typical Soils and Clays.

into the diagram, their velocity values were calculated on the assumption that they obey Stokes' law and that the diameter of particle represented by the upper limit of the silt is $\cdot 01$ mm. The lines thus obtained are *D*, *E*, and *F*, respectively and are inserted for the sake of completeness. By taking into account the coefficient of viscosity and using glycerine-water mixtures, these points on the distribution curve might be obtained by the above method. It is however simpler to use the sieve method to fill in the right hand portion of the curve. It may be added that the data of an ordinary mechanical analysis are given graphically by the difference between successive intercepts on the ordinates *A*, *B*, *C*, etc. For example the fine silt is given by the difference between the intercept on *B* and the intercept on *A*, the silt by the difference between the intercept on *C* and the intercept on *B* and so on.

By suitable choice of depths and times any required degree of detail can be secured in any part of the curve. If a large number of points are required it is convenient to prepare a large volume of suspension and work with a number of separate cylinders. The details may be left to the convenience and ingenuity of individual operators. Possibly a more convenient, though scarcely less expensive, method of sampling may be devised. Any measurable physical property of dispersions which depends on concentration may be considered in this connexion. For very dilute suspensions it is possible that a nephelometric method might be devised.

The method may find its best use as a substitute for the present standard method of mechanical analysis. The suggested procedure in this case is as follows. The air dried sample is treated with $N/5$ hydrochloric acid exactly as in the ordinary method, using however 20 grams instead of 10 grams of soil. The fine gravel and coarse sand are separated in the ordinary way and the finer material passing the 100 mesh sieve is shaken with 600–700 c.c. of water and 50 c.c. of 10 per cent. ammonia in an end over end shaker for two to four hours, the longer period being necessary in the case of soils with much organic matter. After shaking, the suspension is made up to one litre, which is equivalent to 2 per cent., reckoning on the original material. The following determinations are then made successively by the method described above.

Depth cm.	Time hrs. min.	Velocity cm./sec.	Giving percentage of
(a) 30	0 5	0.1	silt + fine silt + clay
(b) 12	0 20	0.01	fine silt + clay
(c) 6	16 40	0.0001	clay
or 7.2	20 0		
or 8.6	24 0		

Lastly to determine the fine sand, the suspension remaining after the last sampling is poured away to about 200 c.c. without shaking up the sediment. The remaining suspension and sediment are then washed into a beaker and the fine sand determined by the ordinary method, using the 10 cm. and 100 seconds, or 7.5 cm. and 75 seconds sedimentation.

The following figures are the results of an actual determination on a Pennant Grit soil from Glamorganshire:

By ordinary method	Fine gravel	4.6 %
	Coarse sand	20.1
	Moisture	4.2
	Organic matter	<u>10.2</u>
	Total	39.1

So that fine sand + silt + fine silt + clay should = $100 - 39.1 = 60.9$ (1).

By new method, using 2 % suspension.

For 5 mins. and 30 cm., weight of ignited material in 20 c.c. = .112 gm.

Therefore concentration = $5 \times .112 = .560$ %.

Therefore silt + fine silt + clay = $\frac{.560 \times 100}{2} = 28.0$ %(2).

Similarly for 20 mins. and 12 cm., ignited material = .065 gm.

Concentration = .325 % and fine silt + clay = $\frac{.325 \times 100}{2} = 16.25$ %(3).

Similarly for 20 hrs. and 7.2 cm. ignited material = .019 gm.

Concentration = .095 % and clay = $\frac{.095 \times 100}{2} = 4.75$ %(4).

Subtracting (4) from (3), fine silt = 11.5 %.

Subtracting (3) from (2), silt = 11.75 %.

Subtracting (2) from (1) fine sand = 32.9 %.

By direct sedimentation at end of experiment, fine sand = 32.5 %.

EFFECT OF VARIATIONS IN CONDITIONS OF WORKING.

In order to form some idea as to the latitude allowable in the conditions of working the following points were investigated.

(a) *Effect of concentration.* A number of determinations were made on a clay suspension of varying concentrations. Results are given for different times and depths.

	Concentration of original suspension	Percentage of original concentration
Depth 6 cm. Time 10 mins.	.50 %	63.0
	.625	61.0
	1.00	62.0
	2.00	63.2
	2.50	64.0
	4.00	63.1
	5.00	65.7

Depth 6 cm.	{	0.50	53.0
		1.00	51.5
		2.00	52.2
Time 100 mins.	{	4.00	49.0
		1.00	45.0
		2.00	44.2
Depth 15 cm.	{	5.00	46.3
		1.00	37.5
		2.00	38.0
Time 240 mins.	{	5.00	41.7
		1.00	
		2.00	
Depth 7 cm.	{	1.00	37.5
		2.00	38.0
		5.00	41.7

The variations in the results obtained with varying concentrations are not serious when the nature of mechanical analysis and the nature of the material under examination is taken into account. In the standard English method the concentration used is about 2 per cent. In view of the small weights to be dealt with in very dilute suspensions and the consequent magnification of weighing errors, it was decided to use 2 per cent. suspensions as a general rule and in the comparisons given later of the results by the new method with those by the old method, this concentration was used.

(b) *Diameter of column.* The diameter of the column may be expected to have some effect on the result obtained since, apart from any boundary effect, the fall in height due to the removal of liquid will be greater in narrow columns. The following experiment serves to test this point.

Clay suspension 2.5 %.	Time 19.5 hrs.	Depth 9 cm.
Diameter of cylinder	Weight of ignited material	
7.7 cm.		0.102 gm.
6.0		0.101
4.6		0.102
3.4		0.098

The last cylinder was an ordinary 250 c.c. measuring cylinder. It would appear that with $\frac{1}{2}$ litre, litre and 2 litre cylinders consistent results may be obtained and that the diameter of the cylinder is immaterial. In general, a litre or $\frac{1}{2}$ litre cylinder was used.

(c) *Equivalence of Depth/Time ratio.* Theoretically the same concentration should be obtained for different times and depths provided that the ratio depth/time, *i.e.* the limiting velocity is constant. This point is investigated in the following experiments.

Kaolin. 2.5 % suspension in 0.025 % sodium carbonate.		
Depth	Time	Weight of ignited
cm.	hours	material
5	5	0.165
19.5	19.5	0.169
Clay. 2.5 % suspension in 0.025 % sodium carbonate.		
4	1	0.254
12	3	0.253
5	18.75	0.178
18	68	0.177

(d) *Errors in Depth of Sampling.* The effect of errors in depth of sampling may be best demonstrated by considering the nature of the vertical concentration gradients in a column of suspension after varying times. A series of curves showing the relation between depth and concentration for different times can readily be derived from the summation curve for the material under consideration. For example if it be known from the summation curve that 50 per cent. of the material has

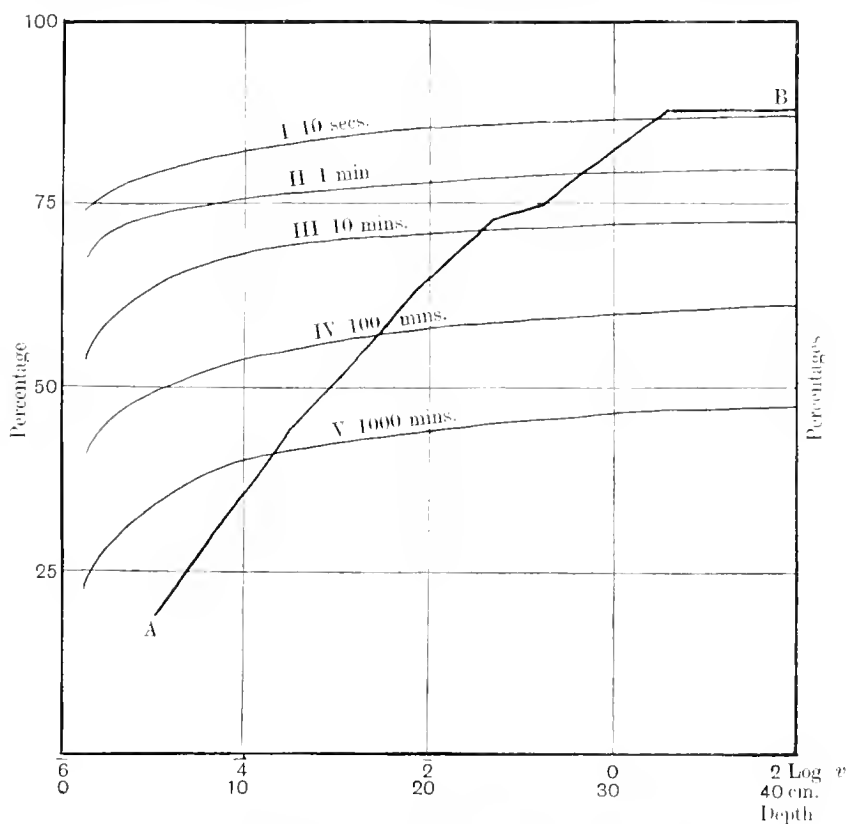


Fig. 4. Concentration Gradients at Different Depths.

a limiting velocity less than .01 cm. per second ($\log v = \bar{2}.0000$), then at depth 10 cm. after 1000 seconds, the concentration will be 50 per cent. of the original concentration. A series of concentration gradients for a clay is shown in Fig. 4. *AB* is the summation curve and the curves I, II, III, IV, and V give the percentages of the original concentration at different depths for 10 secs., 1 min., 10 mins., 100 mins., and

1000 mins. respectively. The $\log c$ abscissae refer of course to the summation curve AB , and the depth abscissae to the concentration curves.

It will be seen that the change in concentration with depth after any given time is very gradual below the first few centimetres. Thus, after ten minutes, the concentration in the case illustrated only changes about 2.5 per cent. of the total concentration between 10 cm. and 20 cm. With longer times the gradient becomes rather steeper. Errors of the order of a few millimetres in depth of sampling have thus very little effect on the concentration obtained. This of course only holds so long as the material under experiment has a fairly smooth summation curve. With a material having an irregular type of curve, depth errors might be more serious.

Similar considerations can be developed for the time and temperature error.

The errors introduced by the above variations in working conditions though scarcely negligible are nevertheless not serious when the character of the material is considered and it may be doubted whether they are of significance from the point of view of the genetics and physical properties of soils and clays. The Odén method is of course less exceptionable from the point of view of delicacy and must be used where critical data are required. The method described in the present paper could of course be made to give results strictly comparable among themselves. It is however desirable to have a method which can admit of some latitude to suit the convenience of individual workers and which has to that extent and within reasonable limits the character of an absolute method.

AGREEMENT WITH RESULTS OBTAINED BY THE STANDARD METHOD.

In view of the enormous number of results accumulated by the older method, it would be a doubtful advance to suggest a new method, even though more convenient and accurate, if the results obtained by it could not be used for comparison with the older results. A considerable number of determinations were therefore made by the new method and the results compared with those already obtained by the old method. In the following table the results of this comparison are given. For convenience of statement only three values are given, namely, clay, fine silt, and silt. The same method of dispersion was used throughout except in the case of certain clays, which contained practically no organic matter and were dispersed in .025 per cent. sodium carbonate.

Table II. *Comparison of Results by Old and New Methods.*

Soil or clay	Clay		Fine silt		Silt	
	Old	New	Old	New	Old	New
A 43 A	9.5	10.8	26.8	26.8	15.0	15.0
A 55 A	10.5	10.1	21.7	21.7	13.1	13.2
F 2 B	29.0	28.7	34.0	34.0	20.9	20.6
D 44 A	3.5	5.9	38.1	38.0	15.8	15.9
G 8	13.3	16.6	24.0	20.0	18.4	18.4
D 10	13.9	13.6	57.6	57.9	9.3	9.2
C 34	3.9	4.0	13.6	13.5	10.0	10.0
D 11	2.7	3.8	14.6	14.4	7.2	7.0
D 73	5.8	2.4	22.6	22.2	13.1	17.4
D 49	2.0	2.2	12.2	9.3	8.2	12.0
Llangoed clay	43.4	48.4	30.7	30.8	3.9	3.9
Ruabon	25.8	23.2	44.4	44.4	17.5	17.4
London clay	40.0	(38.2	22.6	(26.4	11.7	(12.6
		37.2		26.4		13.4
		38.4		27.4		12.4
Kaolin	23.5	(24.2	48.4	(46.8	13.7	(16.2
		23.6		46.4		17.8
		23.8		47.2		15.8
Ab. 4	(11.0	12.1	24.5	28.7	17.7	18.0
		10.5	26.0	26.5	18.7	17.3
		13.0	25.9	26.2	18.5	17.3

The agreement between the two series of results is generally close and not unsatisfactory when it is considered that mechanical analysis by the older method is liable to considerable errors. On the average, putting the figures obtained by the old method as 100, the new method gives 102.7 for the clay, 100 for the fine silt and 99.6 for the silt. It would thus appear that there is no appreciable constant error in comparing results by the two methods. In view of the large number of manipulations required in the old method, there are more occasions for error than in the new, and any serious disagreement is at least as likely to be due to errors in the former as in the latter method.

It will be noticed that the most serious disagreements are in the case of light soils. To secure a perfect comparison it would be necessary to secure that the preliminary dispersion is exactly the same in both methods. In the standard method the material is repeatedly triturated after each pouring off. The method provisionally adopted for dispersion in the new method, namely a 2 to 4 hour shaking in an end over end shaker would appear to give a comparable degree of dispersion. The longer period is apparently necessary in the case of soils rich in organic matter. Dispersion by sodium carbonate¹ gave good results with raw clays but was unsatisfactory with soils containing much organic matter.

¹ Cf. Joseph and Martin, *J. Agric. Sci.* 1921, **11**, 293-303.

CONCLUSION.

The method of mechanical analysis above described has two recommendations. It is more expeditious and economical than the standard method. Granted that the new method gives reliable results, its adoption would remove one of the gravest objections against the present method, namely its laboriousness. With the new method it has been found possible in the writer's laboratory to carry out six mechanical analyses in a day. With proper organisation there should be no difficulty in carrying out 35–40 analyses in a week. Anyone familiar with the routine of the older methods will realise that it would be impossible to deal with such numbers single handed. There is the further consideration of economy in the use of beakers, filters, etc., to say nothing of the distilled water, ammonia and hydrochloric acid required. On the other hand the suggested method requires careful manipulation. Working with 2 per cent. suspensions, an error of 1 mgm. in weighing corresponds to .25 per cent. in the result obtained. It is doubtful if the method would commend itself for teaching purposes.

The new method may be used within limits for obtaining continuous curves such as are obtained by the Odén and Wiegner methods. Provided temperature conditions are controlled it is easily possible to carry the distribution curve for a soil or clay considerably beyond the limits of clay as defined by the standard method, and the constitution of the finer fractions can thus be investigated over ranges hitherto scarcely explored¹. An application may also be found in the investigation of changes in degree of dispersion consequent on manuring, cultivation and season.

With regard to the errors of the method it may be remembered that the experiments recorded were carried out in ordinary measuring cylinders. These are rarely uniform in cross section. With perfectly cylindrical columns, no doubt, better results would be obtained. With regard to temperature effects, no attempt was made to secure rigid control of temperature conditions. As in the ordinary method, the settling took place in an ordinary laboratory with its unavoidable vicissitudes of temperature. Care was however taken to avoid the proximity of sources of heat. In certain cases where the settling columns were in the vicinity of radiating surfaces, unsatisfactory results were obtained. Generally speaking for the longer periods the columns were

¹ A considerable number of clays have, in fact, been followed by this method as far as $\log v = 7$, which appears to be near the lower limit.

put away in a large cupboard in a room without any burners or other sources of heat. The possibility of convection currents was thus avoided as far as possible.

By a suitable choice of depths and times the method could be readily adapted to systems of fractionation other than that in use in this country. In the American systems, where the numbers of fractions separated are large, the amount of work involved and the possibilities of error are serious. The above method might be of use in such cases. By setting out the results as summation curves the mechanical analyses by any system of grading could be obtained by interpolation.

SUMMARY.

(1) The expression of mechanical composition by means of continuous curves is discussed. It is suggested that a convenient representation will be obtained by showing summation percentage as a function of the logarithm of settling velocity.

(2) The effect of a gel coating on the settling velocity of a particle is examined and it is shown that a reduction in velocity takes place which is a simple function of the thickness of the gel coating.

(3) A method is outlined by which the mechanical composition of a soil or clay is derived from determinations of the concentration of a settling suspension for different values of depth/time.

(4) A shortened method for mechanical analysis is described which gives results in good agreement with results obtained by the present standard method.

(5) The effect of various modifications in conditions of working is discussed.

(6) The nature of the concentration gradients in a settling column of a suspension is examined. It is shown that below the first few centimetres the change in concentration with depth is very gradual.

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CONTENTS.

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	PAGE
1. DEIGHTON, THOMAS. Some investigations on the electrical method of soil moisture determination. (With 6 Text-figures.)	207
2. WOODMAN, HERBERT ERNEST. The chemistry of the strength of wheat flour	231
3. IVANOFF, E. I. On the use of artificial insemination for zootechnical purposes in Russia	244
4. CAPSTICK, J. W. and WOOD, T. B. The effect of change of temperature on the basal metabolism of swine. (With 4 Text-figures.)	257
5. HORTON, E. and SALMON, E. S. The fungicidal properties of certain spray-fluids. III	269
6. COLLINS, S. H. and THOMAS, B. The sugars and albuminoids of oat straw	280
7. ROBINSON, GILBERT WOODING. Note on the mechanical analysis of humus soils	287
8. JONES, S. G. A bacterial disease of turnip (<i>Brassica napus</i>). (With Plate III.)	292
9. ROBINSON, GILBERT WOODING. A new method for the mechanical analysis of soils and other dispersions. (With 4 Text-figures.)	306

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TEMPERATURE AND OTHER FACTORS AFFECTING THE QUALITY OF SILAGE.

BY ARTHUR AMOS, M.A.

AND THE LATE GWILYM WILLIAMS, M.A., N.D.A.

(*School of Agriculture, Cambridge University.*)

INTRODUCTION.

IN 1883 Mr George Fry, F.L.S.¹ described a series of observations which he had made upon silage. From these he drew the conclusion that if the conditions of silage making were such that the temperature exceeded 15° C. sweet brown silage resulted, but that if the temperature failed to rise above 10° C. then sour silage with a rather repulsive odour was produced. These results were obtained in the type of silos then commonly in use, which varied in depth generally between 12 and 18 feet, frequently had a considerable surface area and were filled comparatively slowly.

In 1884 Dr Augustus Voelcker, F.R.S.² confirmed Mr Fry's experience with silage in the regulation and maintenance of a proper temperature and mentions 125° F. (51.3° C.) as being the point below which sweet or hay fermentation does not take place. He further stated that sweet silage keeps only a short time on exposure to air, whereas sour silage may keep 6-9 months exposed to air.

In 1886, Dr J. A. Voelcker³ described the making of sweet silage by ensuring the temperature of fermentation rising to 122° F. = 50° C., "the point which Mr George Fry considers must be reached to get sweet silage."

M. Goffart⁴, however, is quoted as follows: "My maize, my green rye, my fodders of every kind have scarcely changed colour after eight or ten months of ensilage." From this it is obvious that the silage of M. Goffart, which had "scarcely changed colour" was very different material from the "sweet brown silage" advocated by Fry.

Babcock and Russell⁵ state that "the popular opinion that good silage can only be made with considerable heat is erroneous." Good silage

¹ *Agricultural Gazette*, Aug. 27th, 1883, Nov. 26th, 1883 and April 14th, 1884.

² Voelcker. *Journal of the Royal Agricultural Society*. 1884.

³ Voelcker. *Ibid.* 1886.

⁴ Silos for British Crops by the sub-editor of the *Field*.

⁵ Babcock and Russell. *Wisconsin Agricultural Experiment Station*, 17th and 18th Annual Report, 1900.

was made by them in small retainers at temperatures which did not exceed 80 F. 27 C.; thus, as the authors say, "disproving Fry's theory, that a temperature of at least 120 F. was essential for good silage."

Many American and other experimenters have obtained similar results, of which may be quoted those of Neidig¹. In this case the maximum temperature recorded at the centre as distinct from the top surface of the silo was 91 F. 33 C., and good silage resulted.

When in 1917 the authors began to study silage-making in the American type of tower silo, agricultural opinion in England still retained the distinction between "sweet" and "sour" silage which Fry had enunciated and did not realize the possibilities of other types of silage. For this reason, as well as for the fact that silage produced in the experimental silo at Cambridge varied very greatly not only from year to year, but also in different parts of the same silo, it became apparent that, before reliable feeding experiments with silage could be conducted, it was necessary firstly to define the different types of silage which were capable of being produced, and secondly to define the conditions under which each type could be produced.

The observations in this paper are divided into two parts. The first part concerns those made upon silage produced by a large number of silage growers in the Eastern Counties and elsewhere, from which a few characteristic examples have been described. The second part describes a more accurate series of observations made in the experimental silo at Cambridge.

In the earlier years the late Mr G. Williams co-operated but his untimely death in 1920 prevented him from helping to complete the work.

QUALITATIVE OBSERVATIONS MADE ON SILAGE PRODUCED BY FARMERS UNDER VARIOUS CONDITIONS.

I. On farms belonging to Mr J. Thistleton Smith, Fakenham, Norfolk.

Mr Thistleton Smith farms light to medium land, upon which the silage crops generally stand well. The crop mixture consists of wheat, oats, and tares, which is allowed to become fairly mature before cutting (the oats being past the milk stage and the tares well seeded). If the crop is succulent it is allowed to wilt a few hours before being ensiled.

¹ Neidig, "Chemical Changes in Silage fermentation." *Iowa Agric. Exp. Station, Research Bulletin*, No. 16.

The resulting silage over a period of years and in several silos has been of a yellowish-brown to brown colour with an acid though quite pleasant smell. The silage has been readily eaten by all classes of stock, which have invariably thriven upon it.

This is much the most common type of silage now being produced in the Eastern Counties of England and appears to be universally produced in tower silos from a mature crop which is reasonably dry when ensiled.

2. In two silos belonging to Mr F. W. D. Robinson, Beccles, Suffolk, 1919-1920.

The crops in this case were oats and tares grown upon light land. They were cut in a medium condition of maturity, the oats being in milk and the tare pods full-grown in length but with immature seeds. The crops were ensiled immediately after cutting. The resulting silage possessed a green colour with a smell which was neither "sweet" nor "sour"; it can best be described as "fresh" and "fruity." Stock ate it greedily and throve upon it.

It would seem highly probable that M. Goffart's "crops of every kind which had scarcely changed colour after 8 or 10 months of ensilage" must have been of this character.

3. On Mr Arnold Oliver's farm at Bures in Suffolk.

In two successive years 1919 and 1920 the silos were filled with oat and tare crops cut in a medium condition of maturity, and under conditions very similar to those prevailing in Mr Robinson's silos.

In each case the crop was ensiled immediately after cutting and produced a green silage with the same "fruity" smell observed with Mr Robinson's silage. In 1920 a maximum thermometer was inserted about the middle of the silo and this recorded 30° C.

4. On General Adlercron's farm at Culverthorpe, near Grantham.

In 1920 the silo was filled during September and October from a late-sown oat and tare crop which was badly laid. The crop had grown to a great length and was semi-rotten close to the ground. Much rain fell during the ensiling process. The resulting silage was dark brown almost black in colour and possessed the most objectionably sour pungent smell. So tenaciously did this smell cling to anything touching it that the writer, who had occasion to handle some, was unable to get the taint from his hands for 36 hours. This silage was eaten by cattle but without relish.

It is satisfactory to record that in the season 1921-22 beautiful silage has been produced on this farm by ensiling under conditions similar to those adopted by Mr J. Thistleton Smith.

5. Silage made by Capt. Nicoll of Alresford in 1920.

The crop was slightly overmature when cut, but not badly laid; during the greater part of the filling the crop was allowed to wilt after cutting and frequently got very wet with rain, but during the last two days of filling the crop was ensiled directly after cutting.

The top part coinciding with the dry period of filling of the silo produced very good silage upon which the cattle thrived, but the bottom coinciding with the wet period of filling was poor silage and the cattle fell away whilst feeding upon it.

In 1921, the silo was filled throughout with freshly cut material and the product was excellent: green in colour with the characteristic "fruity" smell.

6. Silage made from immature crops on Mr Alfred Amos' farm at Wye, Kent.

Maize was grown for several years in succession from 1899 to 1904 and ensiled in a tower silo, but the variety grown—American Horse Tooth—failed to ripen sufficiently for ideal silage purposes, rarely getting beyond the flowering stage. Under these conditions the silage was invariably "sour" with a pungent clinging smell. The cattle ate it, but not greedily and did not thrive greatly upon it.

In 1921, a crop of winter oats which had grown very rankly and was likely to be badly laid before harvest was cut off between May 8th and 10th when a foot to 15 inches high, and put into a clamp silo after wilting for 24 hours. The crop was of course very immature. The resulting silage was of a greenish olive colour with a most objectionable smell, similar to that described in General Adlercron's silage. The silage was fed to dairy cows in late summer, being scattered on the grass during the severest part of the drought in that year. The cows did not eat it greedily until the smell had partially blown away, but after lying in the sun for an hour the silage was readily eaten. The cows kept in good condition, milked well and no taint was noticeable in the milk.

7. Silage made in a stave silo in Sussex in 1920.

The crop consisted of oats and tares in which a large proportion of charlock was growing. This was allowed to become very mature before cutting, so that the charlock had set seeds, which were almost ripe, and produced stems which were hard and woody. The crop after cutting was allowed to wilt 24 to 48 hours and was consequently very dry when ensiled. This fact combined with the woody character of the charlock stems prevented the chaffed crop being adequately packed by trampling, so that much air was included.

When the silo was opened numerous tiny patches of mould were found throughout the whole depth of the silo and of necessity became mingled with the rest when thrown down for feeding. This silage was very dark brown almost black in colour, possessed a strong smell of ammonia and was musty. Cattle, when fed upon it, only ate it under compulsion unless they were able to pick out pieces uncontaminated with mould.

SILAGE AT CAMBRIDGE, 1917-1921.

The silo has been partially or completely filled each of the five years during the period, and in addition a silage stack was made in 1918. Careful records were kept of the crop as ensiled and of the silage as taken out. In many cases moisture content has been recorded by means of weighed samples enclosed in wire netting sample-bags.

Temperature has been recorded by two methods. In the first a hollow iron gas-pipe was driven into the silage after the silo had been filled. A thermometer was then lowered to different levels in the silage, allowed to remain till it had taken up the temperature of the surrounding silage, quickly pulled out and the temperature read off. Readings were made at different depths at daily or longer intervals. The length of the gas pipe was never more than 8 ft. It is obvious, therefore, that the temperatures of the surface 8 ft. only could be ascertained by this plan. The method is open to the further criticism, that the silage is constantly settling; if, therefore, the tube is driven in 8 ft. from the surface one day and the silage settles, the tip of the tube is no longer 8 ft. from the surface. In the observations recorded the tube was driven in to the full depth after trampling the silage at the time of the first reading and was not driven in further as the silage settled. The temperature readings on subsequent days were therefore taken at depths which corresponded approximately with the same layers of silage as those from which the temperature was taken on the first day.

In the second method maximum thermometers were buried in the silo at more or less regular intervals as the filling of the silo proceeded. These were carefully corked within short lengths of iron gas pipe to prevent breakage, and placed just beneath sample bags put in at the same time. The thermometers were recovered as the silage was used and the maximum temperatures recorded.

In 1917 the silo was filled with a crop of oats and tares cut when fairly mature, the oats being well in milk and the tares with full-grown pods and the seeds beginning to dent the pods. The crop, which was

lodged but not badly laid, was cut on July 16th and 17th in dull weather, a quarter of an inch of rain fell on each of July 17th and 18th and interfered with the commencement of filling on the latter day. Filling had to be stopped on July 19th, when the silage cutter broke down.

The percentage of moisture in the green crop as filled to the silo varied from 70·3 per cent. at the bottom when the crop though wilted contained some added rainwater, to only 61·6 per cent. at the top when the crop was wilted and dry.

The following table gives a record of the daily temperature readings on the centigrade scale for ten days after filling, and subsequently at longer intervals of time.

Table 1. 1917 crop.

Date	Temperature at 6 ins. °C.	Temperature at 2 ft. °C.	Temperature at 5 ft. °C.
July 20	26	—	—
" 21	49	33	27
" 22	60	34	29·5
" 23	65·5	35·5	32
" 24	63	40	33
" 25	65·5	46·6	34·5
" 26	62·5	47	35
" 27	64·5	49·5	35
" 29	63·5	48	36·5
" 30	—	49·5	36·5
Aug. 2	—	49·5	36·5
" 5	—	—	37
Oct. 1	—	—	31·5
" 23	—	—	27
Nov. 3	—	—	26·5
" 15	—	—	24·5

Except on July 20th, when the reading was made at 9 a.m., the thermometer readings were taken at or near 5 p.m. After July 29th in the case of the 6 ins. depth and August 5th in the case of the 2 ft. depth, readings ceased to be taken because it was impossible to ascertain the corresponding depth, and moreover temperature changes were only those due to cooling.

When the silo was opened on November 12th and subsequent days it was found that the level at which the 6 in. temperatures had been taken consisted of spoilt mouldy material from which much of the moisture had been driven out by the heat. The range of temperature therefore of 60° to 65° corresponded with moulding of the silage.

The silage taken from the 2 ft. depth, where the temperature rose to 49° C., had a uniform dark brown colour with a characteristic "sweet" pleasant smell similar to that of an overheated hay stack, and was evidently comparable to the "sweet" silage described by Fry in earlier days.

The silage taken from the 5 ft. depth, where the maximum temperature did not exceed 37.5°C ., was of a much paler brown colour with a strong, somewhat acid, flavour, similar to that described in Mr J. Thistleton Smith's silos.

Two feet from the bottom of the silo where doubtless the temperature of fermentation was lower though records were not obtained, the colour was still brown but the smell was much more pungent and very unpleasant, and similar to that described in General Adlercron's silo. The smell was most tenacious and when handled tainted the hands so that even washing with soap and water failed to remove the unpleasant smell for several hours. This silage in contrast with the previous two types was not relished by stock.

In the light of later experience, it seems probable that the chief factor contributing to this condition was the rainfall upon July 17th and 18th, causing a certain amount of decomposition of the green crop in the field, and resulting in some rainwater being conveyed to the silo with this part of the crop.

In 1918 the silo was filled at the bottom with rye and tares and with oats and tares at the top; both crops were autumn sown. The rye and tares stood well whereas the oat and tare crop was somewhat but not badly laid. Cutting commenced on July 1st when the rye was rather old, the grain being full-grown but soft and the glumes dry; the tare seeds were denting the pods which were well developed; the oats were forward in milk. The crop was cut 24 to 48 hours in advance of filling. This continued from July 2nd to July 5th, when the silo was full. It was left over the week-end to settle and refilled on July 8th with oats and tares cut the same day. During the whole period of filling the weather was beautifully sunny and no rain fell.

The following table gives a record of temperatures taken at or about 5 p.m. each day at first at daily and later at longer intervals.

Table II. 1918 *crop*.

Date	Temperature at 1 ft. $^{\circ}\text{C}$.	Temperature at 4 ft. $^{\circ}\text{C}$.	Temperature at 8 ft. $^{\circ}\text{C}$.
July 9	37	32	45
" 10	41	34	42.5
" 11	47	34.5	41
" 12	47.5	35	40
" 13	47	35.5	39.75
" 14	47.5	35.5	39.5
" 16	49	36.25	38.75
" 18	46.5	35.75	38
" 21	45.75	35.5	37.5

The silo was opened on November 10th when it was found that the silage at 1 ft. deep, the level of the first set of temperature readings, was of dark brown colour with a "sweet" pleasant smell in every way similar to that immediately below the top of the silo in the previous year although this part of the silo was filled with freshly cut crop. The maximum temperature recorded at this depth was 49 °C. and the silage contained 72 per cent. of moisture when taken out. It is in fact almost invariably the case when a silo is filled with oats and tares or some similar crop that, after the mouldy surface is removed, a shallow layer of "sweet" silage is found; this, however, in most cases rapidly gives place to silage of different character.

At 4 ft. deep, where the temperature did not exceed 36.25 °C., the character of the silage was of paler brown colour and had a pleasant smelling though acid flavour.

At 8 ft. deep the temperature records are higher than at 4 ft., and starting at the comparatively high figure of 45 °C. on July 9th fall continuously to July 21st. The explanation of this apparent paradox is that the 8 ft. level dipped just below the top layer put into the silo on July 5th. This being easily accessible to air from July 5th to July 8th, during the interval of the filling of the silo, fermented readily and so reached a high temperature before the silo was refilled on the latter day. It is quite probable indeed that 45 °C. was not the true maximum, for some cooling may have occurred before the thermometer was inserted on July 9th. The silage at this depth was similar to that at the 1 ft. level in that it was "sweet" with a dark brown colour, but the crop having been cut a couple of days before filling the silage was much drier.

In this same silo five sample bags were put at regular intervals during filling, and below each bag a maximum thermometer was placed. Table III gives in the first column the number of the bag, in the second the condition of the crop when ensiled, in the third the percentage of moisture in the green crop, in the fourth the maximum temperature, in the fifth the percentage of moisture in the silage, and in the last column the type of silage produced.

Table III. 1919 *crop*.

No. of bag	Material	% of moisture green crop	Maximum temp. °C.	% of moisture silage	Type of Silage
5	Oats and tares, no wilting	71.7	47.5	71.6	Sweet dark brown
4	" " wilted 4 hrs.	71.5	37	75.6	Acid light "
3	" " " 24 "	65.2	35	69.1	" " "
2	" " " 48 "	66.6	31	69.6	" " "
1	Rye and tares, " 24 "	65.4	30	69.8	" " "

Taking bag 5 first, because this was nearest to the top, and consequently taken out first, it was found to be situated within 1 ft. of the surface and so corresponded with the conditions discussed in relation to the silage 1 ft. deep in Table II. The sample contained a fair amount of moisture, nearly 72 per cent., and reached a maximum temperature of $47^{\circ}\cdot5$ C. The silage produced was characteristically "sweet" with pleasant smell and dark brown colour.

The silage in bag 4, which reached a maximum temperature of 37° C., had an acid though pleasant smell. It was situated not far below the level where the break in filling the silo occurred and doubtless for this reason the temperature is above those in samples Nos. 3, 2 and 1.

The silage in bags 3, 2 and 1 was in each case produced from a crop which had been considerably wilted in dry weather and contained only about 65 per cent. of moisture when ensiled. The maximum temperatures were respectively 35° C., 31° C. and 30° C., and in each case a yellowish brown silage resulted with an acid, typically silage, smell. This was pleasant and by no means tenacious like the smell of the silage from the bottom of the silo in the previous year. These samples seem to be typical of silage produced in tower silos from fairly mature crops which are allowed to wilt under dry weather conditions before being ensiled. The silage is not "sweet" in the sense of the earlier writers and neither is it "sour" enough to be unpleasant. It is readily eaten by stock which thrive well upon it.

In 1918 a silage stack was also made from a crop of spring-sown oats and tares. This was cut on July 20th, allowed to wilt for 24 hours, and built into a circular stack 12 ft. in diameter. The stack heated greatly, the maximum temperature in the bottom half of the stack—ascertained by the use of the same hollow gas pipe with thermometer previously described, but thrust horizontally into the stack—proved to be 58° C., whilst that of the top half rose to as much as 75° C. The whole of the stack was composed of sweet silage, for the most part dark brown, but in some places, where the heat was greatest, almost black in colour.

This silage was readily eaten by cattle, but the losses in fermentation only, as ascertained from two sample bags, amounted to 19 and 21 per cent. of the dry weight respectively. So great a loss indicates that silage made at such high temperatures is uneconomical.

In 1919 the silo was filled with a spring-sown oat and tare mixture. Cutting commenced on August 1th and was completed on August 5th. Filling was carried out on August 5th, 6th, 7th and 8th. The crop was

fairly well developed, the oats just passing out of the milk stage and the tares with full-grown pods and half-grown seeds. The crop stood up fairly well, but a slight shower of rain, amounting only to .04 in., fell on the crop on the evening of August 14th after the first day's cutting. The rest of the filling period was fine though dull.

In consequence of the rain on August 14th, the fodder in bags 4 to 5, Table IV, was slightly wetted by rain after cutting, but with the exception of bags 4 and 2, was dry again before ensiling.

Table IV. 1919 crop.

No. of bag	Material	% of moisture green crop	Maximum temp. °C.	% of moisture silage	Type of silage
7	Oats and tares, wilted 3 days	57.3	46	60.6	Sweet dark brown
6	" " " 2 "	67.1	30	71.1	Acid light "
5	" " " 3 "	65.7	30	71.6	" " "
4	" " " 2 "	69.7	34.5	73.6	" " "
3	" " " 2 "	70.1	31	73.8	" " "
2	Oats and tares, wilted 4 days, but wet with rain	72.6	30.5	75.7	Sour dark "
1	Oats and tares, not wilted, wet with rain	79.8	24	83.1	" " "

Table IV is compiled in exactly the same way as Table III.

When the silo was opened it was again found that the topmost bag, No. 7, near the surface, contained "sweet" dark brown silage and this was associated with a maximum temperature of fermentation, taken just below the bag, of 16 °C. It is probable that the maximum temperature within the bag would have been a few degrees above this point.

Bag 6, which had been cut in dry weather and allowed to wilt two days before ensiling reached a maximum temperature of 30 °C. and contained typical light brown acid silage with a pleasant smell.

Bags 5, 4 and 3 which had been slightly wetted with rain after cutting and then left long enough to dry off the rainwater before ensiling, produced silage very similar to that in bag 6.

Bag 2, which contained a small amount of rainwater when ensiled, contained a slightly unpleasantly sour silage and bag 1, which was next to the floor of the silo and was very wet when ensiled, contained the characteristic unpleasantly sour pungent brown silage. The thermometer indicated that the maximum temperature in this case was only 24 °C., due partly to proximity to the floor of the silo and partly to the exclusion of air from the wet material containing as it did 80 per cent. of moisture. It must also be recorded that in the season 1919 the drain in the floor of the silo was intentionally closed so that no drainage was possible. This may have contributed to the souring of the bottom silage.

Table V. 1920 crop.

No. of bag	Material	% of moisture green crop	Maximum temp. "C.	% of moisture silage	Type of silage
7	Oats and tares, no wilting	69.6	35.5	71.5	Acid light brown
6	Oats, tares and beans, wilted 6 hours	68.1	31.5	70	Acid yellow-brown
5	Oats, tares and peas, wilted 6 hours	66	32.5	70.4	Acid yellow-brown
4	Oats and tares, wilted 24 hrs.	70	33	69.7	Acid light brown
3	" " " 48 "	64.8	36.2	62.8	" " "
2	Oats and tares, cut 6 days before filling. Soaked with rain	72.5	31.3	72.9	"Sour" dark brown
1	" " " "	69.1	30.5	71.8	" " "

In 1920 the silo was filled with a crop of autumn sown oats and tares, except for two small quantities of material which contained in addition peas and beans respectively. The crop was well advanced when cut, the oats being just past the milk stage, the tare pods full grown in length, with seeds denting the pods. The pea seeds were full-grown in size though still soft, and the bean seeds were not quite full-grown. Cutting commenced on July 3rd, but much rain fell during the next five days, so that filling was impossible till July 9th. The following Table VI gives the rainfall for the period.

Table VI. Rainfall 1920.

July 4	0.14 in.	July 8	0.44 in.	July 12	nil
" 5	0.56 "	" 9	nil	" 13	nil
" 6	0.54 "	" 10	0.04 "	" 14	0.05 in.
" 7	0.10 "	" 11	0.05 "	" 15	nil

Bag No. 7, which was situated 2 ft. 6 in. below the surface of the silage, was made from the oat and tare crop; this had been ensiled shortly after cutting. The temperature of fermentation was considerable, 35°·5 C., owing to its proximity to the surface and consequent access of air. This silage had a pleasant acid smell and was much relished by the stock.

Bags Nos. 6 and 5, containing beans and peas respectively, mixed with the oats and tares were in each case wilted only six hours, but the crops were dry and fairly mature before cutting, so that their moisture content as filled was low, 68.1 per cent. and 66 per cent. respectively. In each case good silage resulted characterised by a pale yellow-brown colour with a pleasant acid smell; this was greatly relished by the stock, the pea, oat and tare silage being particularly good.

The silage in bag No. 4 was cut on July 12th and allowed to wilt 24 hours; that in bag No. 3 was cut on July 10th, wetted with slight

showers on that day and the morning of July 11th, but the afternoon was dry and sunny so that the crop when ensiled on July 12th was free from rainwater. Each of these produced light brown silage with an acid but not unpleasant smell. None the less it was not so good as that in Nos. 5 and 6.

Bags Nos. 2 and 1 contained material which had been cut on July 3rd, but owing to very wet weather remained in the field till July 9th before ensiling. A certain amount of decomposition occurred in the field, and the crop was ensiled whilst it still contained some rainwater, the total moisture contents being 72.5 per cent. and 69.1 per cent. respectively. The silage from these bags was of the characteristically unpleasant "sour" variety, previously described; it possessed a dark brown colour and a pungent smell which clung to hands or clothes brought into contact with it; the maximum temperatures recorded with these samples amounted only to 30° C. to 31° C. Experimental cattle, when fed upon it, failed to thrive well.

Table VII. 1921 crop.

No. of bag	Material	% of moisture green crop	Maximum temp. °C.	% of moisture silage	Type of silage
2	Oats and tares, wilted 6 hrs.	67.4	31.5	70.6	Green and "fruity"
1	" " not wilted	76.5	24.5	69.7	" "

In 1921 the crop was ensiled in perfect weather, warm and dry, except that on the night previous to the first day's cutting .06 in. of rain fell, so that the material in bag 1 contained a small amount of rainwater. The crop was not very mature, the tares being in full flower and the oats just in milk. Wilting throughout the filling was reduced to a minimum, the crop being cut only a short time before ensiling. Both bags, and in fact the whole of the silage, was of excellent quality. The silage had an olive colour with a tint of green throughout, though in some parts the green was more pronounced than in others. The smell was entirely different from that previously obtained: it had no suggestion of sourness, although it was made from sappy and rather immature material, nor had it any pungent odour, but it possessed a kind of "fruity" smell suggestive of pear drops and combined with this the smell of freshly cut lawn grass. When fed to stock it was ravenously eaten and under experimental conditions has given excellent feeding results¹.

It is to be remarked when such freshly cut material containing large quantities of moisture is ensiled that much juice may be expressed and

¹ The results of this feeding experiment will be published shortly.

lost from wooden silos. The juice may run to waste and after putrefaction produce unpleasant smells in the yard or it may be given to cattle or pigs as a liquid.

CONCLUSIONS.

1. Many different types of silage may be produced from the same crop according to the conditions of ensiling; the characteristics of the resulting silage varying not only in physical and chemical but also in feeding properties.

2. The following types of silage have been differentiated and some of the conditions of their production ascertained.

(a) "Sweet" dark brown silage. This type of silage is produced when the temperature of fermentation rises above 45° – 50° C.; it has not been produced below 45° C. It is frequently produced in stack silage to which air has ready access, but not generally in tower silos except in a shallow layer 6 in. to 2 ft. thick just below the mouldy surface to which air has ready access. This silage has a dark brown colour varying in intensity according to the temperature of fermentation and a sweet pleasant smell resembling that of heated hay. It is readily eaten by stock, but has generally lost a considerable proportion of its food value through excessive heating.

(b) Acid light-brown or yellow-brown silage is produced in tower silos from crops which are moderately mature when cut and allowed to wilt for varying periods according to the initial dryness until the moisture content of the crop approximates 70 per cent. The maximum temperature of fermentation is generally between 30° and 37° C.

This silage has a yellowish brown to brown colour with an acid though pleasant smell probably largely due to acetic acid, the yellowish types being generally the more pleasant.

This type of silage is eaten greedily by stock, which thrive upon it and is to be commended.

(c) Green "fruity" silage is produced in tower silos from crops which are cut in the earlier stages of maturity, from the time of full flower till the seeds are half formed. The crop must also be ensiled soon after cutting. The temperature of fermentation is low and may vary from 22° C. to 34° C. This type has a green to olive green colour with a smell that is neither "sweet" nor "sour," but can best be described as "fresh" and "fruity." It is greedily eaten by stock, which thrive greatly upon it, and Woodman¹ has recently shown that its digestible properties are very high.

Green silage suffers from one practical disadvantage; large quantities

¹ Woodman. This *Journal*, 12, Part II, April 1922.

of juice are liable to drain away from the silage and carry with them soluble food material, which if allowed to accumulate in the yard undergoes fermentation and produces a smelly mass of putrid material. If, however, the juice is collected cattle and pigs readily drink it. This problem is receiving further study.

(d) Sour silage is produced under at least two different sets of conditions. It may be produced from an immature and succulent crop, or it may be produced from a crop which has been cut and then saturated by rain before ensiling, especially when the crop has become laid and partially rotten at the base before cutting.

Sour silage has a dark brown or olive brown colour with a pungent and most unpleasant smell possibly due to butyric acid. Cattle will eat this, but not readily and do not generally thrive upon it.

(e) Musty silage. One case only of this type had been recorded, in which an over-ripe crop containing much charlock was allowed to wilt and become over-dry before ensiling. A number of tiny mouldy centres were produced in which ammonia was generated.

This silage has a dark brown almost black colour and has a musty ammoniacal smell. Stock refused to eat it or ate it only under compulsion.

3. The classification of silages given above is admittedly superficial, but it may serve to pave the way for the chemist, the plant physiologist or perhaps the bacteriologist to produce similar types of silage under well defined conditions and so make the distinctions more absolute. For the successful practice of ensilage in this country it is fundamental that the conditions by which each type of silage may be produced should be accurately known.

4. In the past persons conducting feeding experiments in this country have rarely attempted to define the type of silage used, and conflicting results have been obtained. When it is further noted that the quality of silage at different parts of a silo may be, and frequently is, fundamentally different, it is of the utmost importance that these facts should be recorded in future feeding experiments.

Our acknowledgements are due to the Ministry of Agriculture who have financed this study, to Messrs English Bros. of Wisbech who generously presented the experimental silo and to Prof. Biffen and his farm managers Messrs H. V. Sheringham and N. Langridge who have facilitated the experiments upon the Plant Breeding Farm at Cambridge.

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AN INVESTIGATION INTO THE CHANGES WHICH OCCUR DURING THE ENSILAGE OF OATS AND TARES.

BY ARTHUR AMOS, M.A.
AND HERBERT ERNEST WOODMAN, PH.D., D.Sc.
(*School of Agriculture, Cambridge University.*)

INTRODUCTION.

THE practice of ensilage has been rapidly gaining adherents in the British Isles since Mr George Jaques began to advocate the oat and tare crop for this purpose in 1913. In view of this fact, it becomes increasingly important for the scientist to be in a position to supply the practitioner with facts from which he may be able to calculate the probable economy of the system before investing the necessary capital in a silo. One of us¹ has already made such a tentative calculation based upon a somewhat limited knowledge of those facts. It is the purpose of these experiments to amplify knowledge in one direction, namely, in that of the chemical changes which occur during the ensilage of oats and tares, so that one may know what are the losses and what the gains, if any, in chemical constituents during the process.

One of us² has recently shown that it is possible to produce at least five distinct types of silage from the same crop. It is, therefore, equally important to be able to state definitely which of these occasion the least net loss during ensilage, and to endeavour by controlling the factors concerned to reduce to a minimum the losses in the making of those types of silage which appear most economical.

The experiences gained with maize silage in America and elsewhere, useful though they be, do not bear directly upon the present problem of oat and tare silage, because the chemical composition of the two crops is very different.

Preliminary work was begun in 1918 when the late Mr Gwilym Williams assisted on the chemical side of the work, but in the earlier

¹ Ensilage by Arthur Amos, *Journal of Farmers' Club*, March 1920.

² Amos and Williams, *This Volume*, p. 323.

years progress was limited by the failure of some of the methods of investigation. In the first season high grade linen bags, such as are used by seedsmen in distributing seeds, were used as containers, but proved defective because the fabric perished as a result of the fermentation in the silo. Next fine-meshed galvanised wire netting was tried, but though in the first year the juices of the silage produced no effect upon this material, in subsequent years this was not the case and consequently accurate weighing of the contained sample became impossible. In the experiments now to be described, loosely woven jute bags, made of the same material as commonly employed for chaff bags, were used, and this has proved perfectly suitable. During this preliminary period certain progress was made in chemical methods which have been of use in the present investigation. It has, however, been thought undesirable to publish the earlier results in detail because of the uncertainties above mentioned.

Samples of silage described in this paper were made in two cases from the commercial silo used on the Plant Breeding Farm at Cambridge; in the remainder from three small wooden cylindrical silos, measuring 6 ft. high and 1 ft. in diameter, which have given highly satisfactory experimental results. In some measure experimental silos of this size constitute a new departure: they are free from the objection commonly used against bottles of silage, that the bulk under experiment is so small that any rise in temperature is immediately dissipated, and yet they enable the experimenter to vary at will one factor only, a condition which is difficult indeed to accomplish by varying the position of the sample bags in a large commercial silo.

These small silos were constructed of 1 in. boards, tongued and grooved and kept tightly pressed together by three circular iron hoops which were capable of being easily tightened by suitable screws. The wood was of plain deal, tarred externally for purposes of preservation. The silos were placed in a shady situation and the floor of each consisted of puddled gault clay. At the time of filling metal extensions 2 ft. high made of iron sheeting were fitted to the top of each silo.

The sample bags, as previously stated, consisted of loosely woven jute, measuring 3 ft. 6 in. long by 2 ft. in breadth; these contained about $\frac{1}{2}$ cwt. of green fodder under average conditions of wetness. When filled with fodder these dimensions were considerably contracted, so that in no case were they within 6 ins. of the walls of the silo when placed in position.

Immediately beneath each sample bag was placed a maximum

thermometer encased within a piece of iron gas piping for protection. Babcock and Russell¹ have shown that such fermentations as produce increase of temperature in the silo take place very rapidly and that the maximum temperature is generally reached within four or five days. The use of a maximum thermometer therefore gives a useful indication of the vigour of the fermentation, provided the heat is not rapidly lost by conduction.

The results upon opening proved that good silage was capable of being made in these small silos, and in two out of the three cases the silage differed very slightly, if at all, from that produced commercially; moreover, the wastage due to spoilt material at the top compared favourably with that of commercial practice, and in the case of silage made from freshly-cut, succulent crop, the amount of spoilt material on top measured only 4 in. in thickness.

OBJECT OF THE EXPERIMENTS.

The purpose of the present investigation was two-fold; firstly to determine the effect of varying the moisture content, both by the wilting of the crop after cutting and by allowing rain to fall upon the crop in the field, upon the quality of the silage produced, and so to ascertain how far this factor influences the producing of different types of silage; and secondly to ascertain the nature and magnitude of the chemical changes which occur when these different types of silage are made and to obtain a comparison of the fermentation losses involved in each case, as well as any benefits which may accrue from such fermentation. Further details of the chemical objects of the experiment are given later.

DESCRIPTION OF CROP.

The crop utilised for the experiment was autumn-sown and was seeded at the rate of $1\frac{3}{4}$ bushels of tares and $1\frac{1}{2}$ bushels of grey winter oats per acre. In the dry summer of 1921 there was approximately equal growth of oats and tares at time of cutting, but no separation of the crop was made. The crop used for the samples was cut at recorded times between June 21st and 23rd, at which time the maturity of the crop was perhaps ideal for making hay, but would generally be considered slightly immature for silage. The oats were then well past flower, some being in milk, others having not quite reached this stage; the tares

¹ Babcock and Russell, 17th and 18th Annual Report, Wisconsin Agricultural Experiment Station.

were in full flower with a few pods half an inch in length. The whole crop stood perfectly and was of excellent quality.

The weather at the time of cutting formed part of the long drought in 1921, so that generally soil and crop were very dry, but .06 in. rain fell in the night previous to the commencement of operations on June 21st. June 21st was dull and fine, but June 22nd to June 26th were scorching days. During the night of June 26th a sharp thunder-storm fell, measuring .22 in. of rain, and about half this quantity next day. Samples Nos. 1, 2 and 3 were cut in the early morning of June 21st and were chaffed and ensiled without delay. Thus they contained some added rainwater, as Table I shows, and contained only 23.5 per cent. dry matter when ensiled. Samples Nos. 4 and 5 were cut at the same time, but allowed to wilt for 24 hours before ensiling, and lost moisture so that they contained 35.7 per cent. of dry matter. Sample No. 6 was cut at 8 a.m. on June 23rd—a scorching day—and allowed to wilt six hours before ensiling, whilst Samples Nos. 7 and 8 were cut on June 23rd, dried by wilting in hot sun till June 26th, then wetted by the thunder-storm and finally ensiled on June 27th, when they then contained 37.8 per cent. of dry matter, indicating that they must have been very dry before the rainfall. It is important to note that the crop used in Samples Nos. 7 and 8 showed no sign of rotting or putrefaction as a result of the rain, a condition that not infrequently occurs when a laid crop of oats and tares becomes wet with rain after cutting.

FILLING OF SILOS AND SAMPLE BAGS.

All the fodder was chaffed with a Papee silage cutter before ensiling. Before filling the sample bags and taking a sample of the original fodder, a suitable quantity of chaffed crop was shot down upon a smooth concreted floor and well mixed by turning. From this well-mixed heap a 7 lb. biscuit tin of fodder was filled by taking small handfuls from different parts of the heap. The lid was closed at once and the sample sent down to the laboratory for analysis. The sample bags were immediately filled by taking portions at random from the same heap, tied up, numbered and weighed. Sometimes more than one sample bag was filled from the same chaffed sample when required for immediate use in different silos or different parts of the same silo; in this way some economy was made in the number of control samples to be analysed and the resulting silage samples were more comparable.

In filling the small silos, chaffed oats and tares were first put in until, when well trodden, the height of fodder in the silo was about 2 ft.;

then a nest was hollowed out into which was first put a short length of iron gas tubing containing a maximum thermometer. Immediately on top of the thermometer was placed a sample bag. Then more chaffed fodder was trodden in until the height of the fodder reached 4 ft., at which level a second sample bag was placed with maximum thermometer as before. More chaffed fodder was added and trampled until with the aid of the extensions previously described the top of the silage reached 6 ft. 6 in. or 7 ft. in height. The silo was then left 24 hours to settle, after which about 6 in. of soil was thrown on top for the purpose of excluding air. Eventually the silage settled until at the time of opening the level of the top of the silage was about 5 ft.

Two sample bags put into the big silo were provided with similar maximum thermometers, and in addition a 4 ft. length of stout string was tied at one end to the bag and at the other to a short thatching peg. The men filling the silo were instructed to keep pulling up the peg as long as possible, so that when the silage was being used the peg might come into view before the sample bag and so give warning of the proximity to the sample.

OPENING OF THE SILOS.

The small silos were opened at convenient times during November, by which time all fermentation had long since ceased and the temperature had for the most part cooled down nearly to that of the prevailing air temperature. Notes were made of the condition of successive layers in the silos as these were emptied, and so soon as a bag was exposed it was removed, cleaned and weighed. The bag was then quickly emptied, cleaned and weighed again; the difference between these two weights giving the net weight of contained silage. The silage, emptied from the bag, was then quickly mixed together, a sample taken from this, placed in a tin, and sent down to the laboratory for analysis.

The two sample bags in the commercial silo were retrieved soon after the markers came into view and whilst the general level in the silo was still one to two feet above the bags.

When silo 1 was opened on November 10th, it was found that there was no more than 4 in. of spoilt material at the top of the silo; then 14 in. of good silage was removed before the first bag (bag No. 2) was reached. This silage possessed a distinctly green colour and a pleasant odour with no suggestion of sourness. The aroma can best be described as "fruity." Bag 1 was taken out on November 15th. It was situated

about 2 ft. below bag 2 and possessed the same pleasant "fruity" smell but the colour, though still green, was not quite so good.

Bag 3, which was taken from the same sample of crop as bags 1 and 2, was retrieved from the big silo on March 28th, and when opened was found to contain silage very similar to that taken from silo No. 1. It possessed an olive green colour and a pleasant, slightly "fruity" smell.

Table 1.

No. of bag	Small silo 1		Comm. silo	Small silo 2		Comm. silo	Small silo 3	
	1	2		4	5		7	8
Interval between cutting and ensiling (hours)	0	0	0	24	24	6	96	96
Initial temperature (°C.)	13	13	13	16.5	16.5	16.5	14.5	14.5
Maximum temperature (°C.)	24.5	22.5	24.5	23.5	24.5	34.5	32.5	39.0
% moisture as ensiled	76.53	76.53	76.53	64.28	64.28	67.40	62.24	62.24
% moisture after ensiling	76.65	75.56	69.31	66.17	66.25	70.07	63.46 (a)	62.06* (b) 60.50
Dry matter as ensiled (ozs.)	247.4	262.4	262.9	334.0	323.6	287.8	293.8	294.6
Dry matter after ensiling (ozs.)	244.6	227.3	232.9	306.5	294.3	267.0	264.2	245.9
% loss of dry matter	13.2	13.4	11.1	8.2	9.1	7.2	10.0	16.5

* (a) Unspoilt sample. (b) Spoilt sample.

An examination of Table 1 shows that the conditions of silage in samples 1, 2 and 3 are very similar, except in one respect. They were ensiled at the same initial and maximum temperatures, which latter was very low, the quality of silage was very similar, and the percentage loss of dry matter was rather high in all cases. The one condition which varied greatly was the percentage of moisture in the final product; this in the two samples in the small silo remained practically the same as in the original crop, whereas in the big silo the percentage of moisture fell from 76.5 per cent. at the beginning to 69 per cent. at the end, indicating that the superimposed weight of silage in the tall silo had caused some of the moisture to be expressed and this drained away.

The drainage liquid was not analysed, but similar drainage in other years has contained varying quantities, between 4 and 10 per cent., of soluble material and is therefore an important source of loss during ensilage whenever it occurs.

As before mentioned, the percentage loss of dry matter in each of these three samples was high, namely, 13.2 per cent. and 13.1 per cent. in the small silo, and 11.1 per cent. in the large silo.

There seems no very obvious reason to account for the greater loss of dry material from the small silo than from the similar sample in the large silo, especially in view of the fact that little, if any, water drained

from the former whilst drainage must have been considerable from the latter.

Silo No. 2 was opened on November 17th: it had been filled from a crop which had been cut 24 hours before ensiling, and was consequently much drier when ensiled, containing only 64.3 per cent. of moisture. There were 8 or 9 in. of spoilt mouldy material on top, doubtless due to the drier crop being more difficult to press down by the soil covering for the exclusion of air. The silage in bags Nos. 4 and 5 contained in this silo was practically identical. It was olive brown in colour with no trace of the green colour found in bags 1, 2 and 3, and had no trace of the "fruity" smell associated with these. The smell, on the other hand, though pleasant, was distinctly acidic and was characteristic of much commercial silage made from similar crops under similar conditions. The rise in temperature as recorded by the maximum thermometers was again low, rising only from $16^{\circ}\cdot5$ C. to $23^{\circ}\cdot5$ C. and $24^{\circ}\cdot5$ C. in each case. This is lower than the temperature commonly recorded with similar silage, for which the maximum temperature is normally about 30° C. In both cases the moisture percentage increased during ensilage, a result occasioned by the disappearance of some of the dry matter during the process, and not by addition of water, for the silos were kept covered to protect from rainwater.

Finally the loss of dry matter during ensilage in this case was considerably less than in the first three samples, a fact which suggests that drying the crop for 24 hours before ensiling is desirable. It should, however, be remembered that when this is done two other sources of loss in the field may be occasioned, namely, that due to respiration and to the breaking off of the leaves as the crop dries.

Bag 6 contained a sample which was allowed to dry for six hours only on a scorching hot day after cutting. It contained only 67.4 per cent. of moisture when ensiled, but this increased to 70 per cent. in the silo, partly by loss of dry matter during fermentation, and possibly also owing to absorption of moisture from other layers in the silo which were moister. In this case the maximum temperature rose to $34^{\circ}\cdot5$ C., and yet the loss of dry matter was only 7.2. The silage produced under these conditions had a pale brown colour with just a suggestion of green about it, and possessed a smell which was faintly fruity with little or no acidic smell. It was by no means typical silage and was characteristic only of a very small layer in the silo. Possibly this represented a transition stage between the green "fruity" silage below and the brown acidic silage about this layer in the silo.

Small silo No. 3 had been filled from a part of the crop which was cut on June 23rd and left in the sun to dry during June 23rd, 24th and 25th, by which time it was three parts made into hay. However, during the night of June 25th and 26th, a thunderstorm occurred, which precipitated 2.2 in. of rain and wetted the crop considerably. June 26th was dull and about 1.0 in. of rain fell. On June 27th a part of the crop was carted in early morning and ensiled in one of the small silos. This crop, in spite of the rain on the previous day, contained only 62.2 per cent. of moisture when ensiled.

The silo was opened on November 29th, when it was found that a considerable depth, about 18 in., of spoilt mouldy material had to be removed from the top before the good silage was reached; even at this depth the silage next the walls was mouldy and occasional spots of mould were found throughout the whole mass. This mouldiness was due in part probably to infection in the field as a consequence of the rain, and in part also to the fact that owing to the dryness of the material it could not be packed so tightly in the silo.

When bag No. 8, situated in the top half of the silo, was removed it was found that the maximum thermometer immediately below it had recorded a temperature of 39° C. Probably the temperature in the bag itself would have been a few degrees higher. The contents of this bag were partly good and partly mouldy; approximately two-thirds were good. This had a yellow-brown colour and an aroma very similar to that of heated hay. It was in fact "sweet" silage. The loss of dry matter in fermentation was high, 16.5 per cent. This was due in part to the excessive heat of fermentation and in part to the action of the moulds present.

Bag No. 7 at the bottom part of this silo contained a few small spots of mould. It had reached a maximum temperature of 32.5° C. It had a yellow-brown colour with a very slight indistinctive smell, with no suggestion of acid to the nose. It appeared different from any type of silage commonly met in practice. The loss of dry matter during fermentation amounted only to 10 per cent.

From experience gained from other sources it appears that the most desirable type of silage is the green "fruity" silage produced in bags 1 and 2 in the small silo, and bag 3 at the bottom of the large silo. This type of silage seems to be produced when a fresh green crop in an early stage of maturity (soon after flowering) is ensiled, with little or no previous wilting, at a low temperature, at or about 25° C. The results of these experiments suggest that with this type a considerable loss of

dry matter may result from the process, especially if much drainage of sap occurs. Bag No. 6 at the top of the large silo produced a silage bordering upon this type, and was ensiled under somewhat similar conditions as to freshness—it was cut only six hours before ensiling—but the maximum temperature recorded in this bag probably accounts for the alteration in type.

The acid brown type of silage produced in bags 4 and 5 in the second small silo is characteristic of much commercially made silage, and is also a valuable type of silage. This is made generally from a moderately dry crop, dried either by wilting or by allowing the crop to mature and so become drier before cutting. The analytical figures show a remarkably low loss of dry matter in these two samples, only 8.2 per cent. and 9.1 per cent. The small loss is certainly a point in favour of this type of silage.

The silage in small silo No. 3 is not typical of commercial silage and therefore no important conclusions can be drawn except perhaps that rain washed fodder as well for its tendency to become mouldy, as for the washing of food material from it by rain in the field, cannot be expected to produce first class silage.

METHODS OF ANALYSIS.

The analysis of the green crop and silage samples was carried out in the following manner. The percentage of dry matter was determined by drying down representative samples of 200 grms. to constant weight in the steam oven. The estimation was carried out in duplicate and the dry residues were finely ground in a mill, allowed to air-dry for several days and then submitted to complete analysis. This involved determinations of moisture, crude, true and pepsin-HCl soluble protein, "amides," ether-extract, crude fibre, ash and nitrogen-free extractives. The results in every case were calculated to dry matter. When calculating the amount of ether extract in the silage samples, it was necessary to make a correction for the volatile organic acids which are lost during the process of drying down.

The analysis of the samples was left in the hands of Mr F. J. Aylett, to whom the writers would like to express their thanks for his careful work in this connection.

In order to gain a further insight into the nature of the changes which occur during the ensilage of green forage, analyses were also carried out on aqueous extracts of the green crop and silage samples. Representative 200 grms. samples of the material were weighed out into

wide-necked bottles of about 1200 c.c. capacity. To the material was then added 500 c.c. of distilled water (previously boiled and cooled) and the bottle was stoppered with a rubber bung and vigorously shaken in a shaking machine for four hours. The resulting dark brown coloured extract was filtered first through muslin, the residual material being well squeezed out, and then through a filter paper. 150 c.c. of the aqueous extract was then made up to 500 c.c. with alcohol; this caused the separation of a small amount of precipitate, which settled readily. The clear liquid, which was slightly yellow in colour, was then filtered off through a dry filter paper and submitted to analysis by means of the Foreman alcohol titration method.

The amounts given in the following description of the method refer to the analysis of the silage extracts; the determinations on the green crop extracts were carried out on larger volumes, since the ingredients to be estimated were only present in relatively small amounts in such extracts.

10 c.c. of the alcoholic liquid were diluted with 50 c.c. alcohol and the solution was titrated with $N/10$ NaOH in the presence of phenolphthalein. When the neutral point was reached, 12 c.c. of a neutral aqueous formalin solution were added, whereon the pink colour was discharged and the addition of one or two drops of $N/10$ NaOH was necessary to restore neutrality. The total titre of $N/10$ NaOH gave a measure of the total acid groups, free and combined, in the extract. It is important to remember that amino acids and amides of the asparagine type are also included in this measurement. The small amount of alkali requisite to restore neutrality after the addition of formalin has been shown by Foreman to be an indication of the amount of dibasic amino acids and proline present in the solution.

The titration method of Foreman also enabled the amounts of amino acids and volatile bases in the extracts to be determined. To 50 c.c. of the alcoholic liquid in a 500 c.c. distillation flask was added an amount of $N/10$ NaOH sufficient to produce neutrality. The contents of the flask were then submitted to steam distillation, a vigorous current of CO_2 -free steam being passed through for about five minutes. The bases with the alcohol were caught in 10 c.c. $N/10$ HCl which had previously been pipetted into the receiver. The excess of acid in the contents of the receiver was determined by titration with $N/10$ NaOH in the presence of alizarin and the amount of acid equivalent to the volatile bases estimated by difference. The liquid remaining in the flask, which is alkaline after the distillation owing to the hydrolysis of the salts of

amino acids, was cooled, diluted with distilled water and titrated with $N/10$ HCl to phenolphthalein, the amount of free alkali in the flask being equivalent to the amino acids in the original 50 c.c. of alcoholic liquid¹.

In order to obtain the amount of volatile organic acids in the alcoholic liquid, 50 c.c. were pipetted into a 500 c.c. distillation flask and an amount of $N/10$ H_2SO_4 slightly in excess of the equivalent to the volatile bases was added. The contents of the flask were then submitted to steam distillation, in every case 500 c.c. of distillate being collected. The acidity of the distillate was determined by titrating with $N/10$ NaOH to phenolphthalein.

The results thus obtained, in conjunction with the moisture content of the material, enabled the amounts of the different ingredients in the original material to be calculated. The results were expressed in the following manner:

	c.c. N
Total acidic groups, free and combined	A
Amino acids and amides of asparagine type	B
Total organic acids of lactic and acetic type	A-B
Organic acids volatile in steam	C
Non-volatile organic acids	A-B-C
Volatile bases	D

Several advantages attach to the use of the above method of dealing with silage extracts which are not possessed by the titration methods previously in use.

(1) The titration of total acidity is carried out in a liquid which is almost water clear, the light yellowish brown colour of the alcoholic liquid practically disappearing on further dilution with alcohol. The end point in the titration is much more satisfactory than that obtained when direct titration of diluted aqueous extracts is resorted to.

(2) On treating the aqueous extract with alcohol as described above, proteins, albuminoses, etc. are precipitated. This treatment therefore removes certain classes of substances which might interfere with the determination of the simpler constituents.

(3) The determinations of the amino acids and volatile bases are carried out in one operation. A knowledge of the relative amounts of these constituents is of value in studying the degree of putrefaction which may have occurred in samples of spoilt silage².

(4) Alcoholic silage extracts prepared in the manner described can be kept over long periods without undergoing change.

¹ For a full account of the technique and significance of the Foreman method, the original publication should be consulted (Foreman, *Bioch. J.* **14**, 451, 1920).

² Foreman and Graham-Smith, *J. Hygiene*, **16**, 109, 1917.

Table II. *Showing changes in content of dry matter, volatile and non-volatile organic acids, amino acids and volatile bases undergone by the oat and tare crop in the various silos per 1000 gms. dry oats and tares.*

Silo I.

	Green oats and tares c.c. N	Bag 1 silage c.c. N	Bag 2 silage c.c. N
Volatile organic acids	9.0	299.6	194.9
Non-volatile organic acids	337.0	923.2	986.6
Amino acids	75.0	172.5	433.1
Volatile bases	17.0	116.4	108.3
Dry matter	1000 gms.	868.5 gms.	866.2 gms.

Silo II.

	Green oats and tares c.c. N	Bag 4 silage c.c. N	Bag 5 silage c.c. N
Volatile organic acids	7.0	268.9	284.7
Non-volatile organic acids	309.0	601.1	534.8
Amino acids	55.0	444.2	493.9
Volatile bases	19.0	89.0	90.0
Dry matter	1000 gms.	917.7 gms.	909.5 gms.

Silo III.

	Green oats and tares c.c. N	Bag 7 silage c.c. N	Bag 8 silage		
			Unspoilt c.c. N	Spoilt c.c. N	Total c.c. N
Volatile organic acids	6.0	121.4	113.1	9.2	122.3
Non-volatile organic acids	243.0	503.6	268.1	69.8	337.9
Amino acids	43.0	288.7	174.1	69.8	243.9
Volatile bases	23.0	84.5	63.5	56.4	119.9
Dry matter	1000 gms.	899.3 gms.	516.5 gms.	318.5 gms.	835.0 gms.

Big silo.

	Green oats and tares		Oat and tare silage	
	Bag 3 c.c. N	Bag 6 c.c. N	Bag 3 c.c. N	Bag 6 c.c. N
Volatile organic acids	9.0	6.0	215.4	304.2
Non-volatile organic acids	337.0	247.0	900.2	761.5
Amino acids	75.0	37.0	365.2	437.8
Volatile bases	17.0	14.0	106.3	92.8
Dry matter	1000 gms.	1000 gms.	886.0 gms.	927.5 gms.

Table III. *Constituents of silage extract expressed as percentages of moisture-free silage.*

No. of bag...	1	2	3	4	5	6	7	8	
	%	%	%	%	%	%	%	Unspoilt %	Spoilt %
Volatile organic acids*	2.07	1.35	1.46	1.76	1.88	1.97	0.81	1.31	0.17
Non-volatile org. acids†	6.53	7.22	6.11	3.11	2.51	5.17	2.85	2.48	—
Amino acids‡	4.76	4.38	3.61	4.24	4.75	4.13	2.81	2.95	1.92
Volatile bases‡	1.17	1.09	1.05	0.85	0.87	0.88	0.82	1.08	1.55

* Calculated as acetic acid.

† Calculated as lactic acid (see table given later under "Changes suffered by Ether Extract").

‡ Calculated as crude protein.

Comments on Tables II and III. The significance of the above data will be dealt with fully in the general discussion of the results. At this point, it is only desired to call attention to the following:

1. In all the bags containing normal silage, the non-volatile acids preponderated largely in amount over the volatile organic acids.

2. Where moulding of the silage occurred (as in bag 8), the volatile and non-volatile organic acids were not simply neutralised by the basic decomposition products of the protein constituent, but were actually destroyed. This is in agreement with the observations of Dox and Neidig¹ and the behaviour is further exemplified by the results of an analysis carried out on a sample of mouldy silage from the top of silo II:

	c.c. N
Volatile organic acids	—
Non-volatile organic acids	25.9
Amino acids	10.7
Volatile bases	16.0

In this case, all the volatile organic acids and most of the non-volatile had disappeared as a result of mould activity. The meaning of the high ratio of volatile bases to amino acids will be discussed later.

3. The results for bag 3 silage are, as anticipated, quite normal, although this bag was allowed to remain in the big silo some months after the other bags had been removed.

¹ Dox and Neidig, *Research Bulletin*, No. 10, Iowa Experiment Station.

Table IV. *Amounts of constituents of green oats and tares and oat and tare silage contained in bag 1 and bag 2 (silo 1).*

Analysis of samples (calculated to dry matter).

	Green oats and tares "o"	Bag 1 silage "o"	Bag 2 silage "o"
Crude protein	12.15	12.27	12.61
Ether extract*	3.59	3.12	3.21
N free extractives	50.67	47.92	47.62
Crude fibre	25.10	27.75	27.87
Ash	8.49	8.94	8.69
True protein	9.20	5.03	5.76
"Amides"	2.95	7.24	6.85
Pepsin-HCl soluble protein	10.02	9.43	9.90
Protein digestion coefficients (<i>in vitro</i>)	82.5	76.9	78.5

* Not taking into account the volatile organic acids of the silage.

	Bag 1			Bag 2		
	Green oats and tares oz.	Oat and tare silage oz.	"o" increase or loss	Green oats and tares oz.	Oat and tare silage oz.	"o" increase or loss
Moist material	1053.0	919.0	- 12.7	1118.0	930.0	- 16.8
Dry matter*	247.1	214.6	- 13.2	262.4	227.3	- 13.4
Organic matter*	226.1	195.8	- 13.4	240.1	207.8	- 13.5
Crude protein	30.0	25.8	- 14.0	31.9	28.3	- 11.3
Ether extract*	8.9	10.9	- 22.5	9.4	10.2	+ 8.5
N-free extractives	125.2	100.8	- 19.5	133.0	106.8	- 19.7
Crude fibre	62.0	58.3	- 5.9	65.8	62.5	- 5.0
Ash	21.0	18.8	- 10.5	22.3	19.5	- 12.5
True protein	22.7	10.6	- 53.3	24.2	12.9	- 46.7
"Amides"	7.3	15.2	+ 108.2	7.7	15.4	+ 100.0
Pepsin-HCl soluble protein	24.8	19.8	- 20.1	26.3	22.2	- 15.6

* Allowance made for silage volatile organic acids as acetic acid. Amount of silage dry matter in bags calculated as residue after drying at 100° C.: 210.3 oz. (bag 1) and 224.3 oz. (bag 2).

Table V. *Amounts of constituents of green oats and tares and oat and tare silage contained in bag 4 and bag 5 (silo II).*

Analysis of samples (calculated to dry matter)

	Green oats and tares %	Bag 4 silage %	Bag 5 silage %
Crude protein	11.37	12.81	13.09
Ether extract*	3.95	3.18	3.15
N-free extractives	49.93	48.62	47.10
Crude fibre	27.03	26.81	27.76
Ash	7.72	8.58	8.90
True protein	8.89	6.75	6.93
"Amides"	2.48	6.06	6.16
Pepsin-HCl soluble protein	9.35	9.97	10.57
Protein digestion coefficients (<i>in vitro</i>)	82.2	77.8	80.7

* Not taking into account the volatile organic acids of the silage.

	Bag 4			Bag 5		
	Green oats and tares oz.	Oat and tare silage oz.	% increase or loss	Green oats and tares oz.	Oat and tare silage oz.	% increase or loss
Moist material	935.0	906.0	- 3.1	906.0	872.0	- 3.7
Dry matter*	334.0	306.5	- 8.2	323.6	294.3	- 9.1
Organic matter*	308.3	280.7	- 8.9	298.6	268.6	- 10.0
Crude protein	38.0	38.6	+ 1.6	36.8	37.8	+ 2.7
Ether extract*	13.2	14.9	+ 12.9	12.8	14.5	+ 13.3
N-free extractives	166.8	146.4	- 12.2	161.6	136.1	- 15.8
Crude fibre	90.3	80.8	- 10.5	87.4	80.2	- 8.2
Ash	25.7	25.8	+ 0.4	25.0	25.7	+ 2.8
True protein	29.7	20.3	- 31.7	28.8	20.0	- 30.6
"Amides"	8.3	18.3	+ 120.5	8.0	17.8	+ 122.5
Pepsin-HCl soluble protein	31.2	30.0	- 3.9	30.3	30.5	+ 0.7

* Allowance made for silage volatile organic acids as acetic acid. Amount of silage dry matter in bags calculated as residue after drying at 100° C.: 301.3 oz. (bag 4) and 288.9 oz. (bag 5).

Table VI. *Amounts of constituents of green oats and tares and oat and tare silage contained in bag 3 and bag 6 (big silo).*

Analysis of samples (calculated to dry matter)

	Green oats and tares		Oat and tare silage	
	Bag 3 oz.	Bag 6 oz.	Bag 3 oz.	Bag 6 oz.
Crude protein	12.15	10.62	10.99	11.56
Ether extract*	3.59	3.38	3.37	3.05
N-free extractives	50.67	52.27	50.65	50.88
Crude fibre	25.10	25.94	26.81	26.20
Ash	8.49	7.79	8.18	8.31
True protein	9.20	8.12	5.14	5.03
"Amides"	2.95	2.50	5.85	6.53
Pepsin-HCl soluble protein	10.02	8.77	8.29	9.01
Protein digestion coefficients (<i>in vitro</i>)	82.5	82.6	75.4	77.9

* Not taking volatile organic acids of silage into account.

	Bag 3			Bag 6		
	Green oats and tares oz.	Oat and tare silage oz.	% increase or loss	Green oats and tares oz.	Oat and tare silage oz.	% increase or loss
Moist material	1120.0	759.0	-32.1	883.0	892.0	+ 1.0
Dry matter*	262.9	232.9	-11.4	287.8	267.0	-7.2
Organic matter*	240.6	214.1	-11.0	265.4	245.2	-7.6
Crude protein	31.9	25.2	-21.0	30.6	30.3	-1.0
Ether extract*	9.5	11.0	+ 15.8	9.7	13.1	+ 35.1
N-free extractives	133.2	116.3	-12.7	150.5	133.2	-11.5
Crude fibre	66.0	61.6	-6.7	74.6	68.6	-8.0
Ash	22.3	18.8	-15.7	22.4	21.8	-2.7
True protein	24.2	11.8	-51.2	23.4	13.2	-43.6
"Amides"	7.7	13.4	+ 74.0	7.2	17.1	+ 137.5
Pepsin-HCl soluble protein	26.3	19.0	-27.7	25.2	23.6	-6.3

* Allowance made for silage volatile organic acids as acetic acid. Amount of silage dry matter in bags calculated as residue after drying at 100° C.: 229.6 oz. (bag 3) and 261.8 oz. (bag 6).

Table VII. *Amounts of constituents of green oats and tares and oat and tare silage in bag 7 and bag 8 (silo III).*

Analysis of samples (calculated to dry matter)

	Green oats and tares %	Bag 7 silage %	Bag 8 silage	
			Unspoilt %	Spoilt %
Crude protein	11.97	13.34	12.68	15.55
Ether extract*	3.87	2.81	2.70	2.88
N-free extractives	45.43	44.49	46.15	38.59
Crude fibre	30.14	30.16	29.82	31.32
Ash	8.59	9.20	8.65	11.66
True protein	8.32	7.46	7.19	11.73
"Amides"	3.65	5.88	5.49	3.82
Pepsin-HCl soluble protein	10.10	10.47	9.62	9.62
Protein digestion coefficients (<i>in vitro</i>)	84.4	78.5	75.9	61.9

* Not taking volatile organic acids of silage into account.

	Bag 7			Bag 8				
	Green oats and tares oz.	Oat and tare silage oz.	% increase or loss	Green oats and tares oz.	Un- spoilt silage oz.	Spoilt silage oz.	Total oz.	% increase or loss
Moist material	778.0	723.0	7.1	780.0	401.0	238.0	639.0	-18.1
Dry matter*	293.8	264.2	10.0	294.6	152.1	93.8	245.9	-16.5
Organic matter*	268.6	240.1	10.6	269.3	139.1	82.9	222.0	-17.6
Crude protein	35.2	35.0	0.6	35.3	19.0	14.6	33.6	-4.8
Ether extract*	11.4	9.4	17.6	11.4	6.0	2.7	8.7	-23.7
N-free extractives	133.5	116.6	12.7	133.8	69.3	36.2	105.5	-21.1
Crude fibre	88.5	79.1	10.6	88.8	44.8	29.4	74.2	-16.5
Ash	25.2	24.1	4.4	25.3	13.0	10.9	23.9	-5.6
True protein	24.5	19.6	-20.0	24.5	10.8	11.0	21.8	-11.0
"Amides"	10.7	15.4	+44.0	10.8	8.2	3.6	11.8	+9.3
Pepsin-HCl soluble protein	29.7	27.4	-7.7	29.8	14.5	9.0	23.5	-21.1

* Allowance made for silage volatile organic acids as acetic acid. Amount of silage dry matter in bag 7 calculated as residue after drying at 100° C.=262.1 oz. In bag 8: 150.2 oz. (unspoilt) and 93.8 ozs. (spoilt).

DISCUSSION OF RESULTS.

1. *Moisture and dry matter changes during ensilage.*

Number of bag	1	2	3	4	5	6	7	8
Change of moisture content (oz.)	-101.5	-152.9	-331.0	-1.5	4.7	+29.8	-25.4	-92.3
Percentage change of moisture content	-12.6	-17.9	-38.6	-0.2	-0.8	+5.0	-5.2	-19.0

The above figures are of interest owing to the fact that certain of the soluble constituents of the silage are lost in the juice draining away from the silo. The losses in the silo are obviously compounded of two main factors: 1. Fermentation losses. 2. Losses in the juice draining away. Thus, where excessive drainage occurs, the percentage loss of dry matter may be high.

Such cases are furnished by bags 1 and 2 (silo 1) and bag 3 (big silo). The reasons for the large losses of moisture from these bags are twofold: 1. The green fodder carried an appreciable amount of superficial moisture owing to its having been rained on just previous to cutting. 2. The oats and tares were cut in an immature condition and were as a consequence exceedingly "sappy." No wilting was allowed to take place before filling the material into the bags and the moisture content was therefore much higher than that of the material in bags 4, 5, 7 and 8.

It is clear that the relatively high losses of dry matter from bags 1 and 2, as compared with those from bags 4 and 5, are to be attributed to the extra losses occasioned by drainage. It follows, therefore, that whilst green "fruity" silage of an excellent quality was obtained by preserving the unwilted, immature forage, yet the excessive drainage, consequent on "sappiness," led to a needlessly high percentage loss of dry matter.

A study of the figures obtained for bags 4 and 5, which were filled with wilted oats and tares and from which little or no juice was lost by drainage, indicate that the actual fermentation losses need not exceed 8.9 per cent. of the original dry matter present.

The large loss of moisture which occurred in bag 3 has already been referred to in an earlier section of the paper. The gain of moisture in bag 6 is explained by the fact that the bag was placed in the big silo and was surrounded by material possessing a higher moisture content. The infiltration of juice has to some extent augmented the amount of dry matter present in the bag, so that the net loss of dry matter as a result of fermentation is low, namely 7 per cent.

It is of interest to note the high percentage loss of dry matter which occurred as a result of spoiling in bag 8, amounting to 16.5 per cent.

II. Changes suffered by the nitrogenous constituents.

It is obvious that during the conversion of the green crop into silage a profound change takes place in the character of the nitrogenous constituents, mainly resulting in the splitting up of a large proportion of the true protein into simple soluble products of the amino acid type. For example, only about 11 per cent. of the crude protein in the bag 1 silage was in the form of true protein, whereas the true protein in the green oats and tares represented 76 per cent. of the total nitrogenous constituents. The figures in this connection for the material in the different bags are as follows:

Number of bag	1	2	3	4	5	6	7	8	
								Unspoilt	Spoilt
Amount of true protein expressed as percentage of crude protein	75.7	75.7	75.7	78.2	78.2	76.5	70.0	70.0	70.0
(Green oats and tares)									
(Oat and tare silage)	41.0	45.7	46.8	52.7	52.9	43.5	56.0	56.7	75.4

The hydrolytic changes affecting the true protein constituent appear to proceed to the greatest extent during the ensilage of the moist unwilted oats and tares. Thus in bags 1, 2 and 3, containing unwilted material, the change caused a disappearance of roughly 50 per cent. of the true protein, this being associated with an increase in the amount of "amides" of about 100 per cent. in the case of bags 1 and 2 and 74 per cent. in the case of bag 3. A bigger increase in the amount of "amides" would undoubtedly have been registered had it not been for the loss of soluble nitrogenous constituents in the large volumes of juice draining away from these bags, since in bags 4 and 5, containing wilted oats and tares, and where little or no drainage occurred, a splitting up of 30 per cent. of the true protein was accompanied by a 120 per cent. increase in the amount of "amides." The slightly wilted material in bag 6, which was converted into silage possessing a high moisture content compared with that of the silage of bags 4 and 5 and which suffered no losses on account of drainage, suffered a large loss of true protein (44 per cent.) and the "amides" were augmented to the extent of 138 per cent.

The material filled into bags 7 and 8 had obviously been subject to change during the time it lay out in the field, since only 70 per cent. of the crude protein was in the form of true protein. The changes in the character of the nitrogenous constituents were not nearly so far reaching in these bags as with the material in the other bags. In bag 7, only a fifth of the true protein was hydrolysed and the "amides" were only increased by 44 per cent. In bag 8, where extensive spoiling occurred, only 11 per cent. of the true protein disappeared and the "amides" were only augmented to the extent of 9 per cent. In the actual spoilt portion of bag 8 silage, the true protein formed a larger proportion of the total crude protein than was the case in the original oats and tares placed in the bag, the rotting of the material having to a large extent used up the "amides."

The above results indicate therefore that the ensiling of "sappy" unwilted forage leads to conditions which are favourable to the extensive splitting up of true protein into "amides." From the nutritive standpoint, it is uncertain whether this can be considered advantageous.

By combining the results obtained in the analysis of the dry matter of the samples with those obtained in the titration of the extracts, it is possible to ascertain in what form the "amide" fractions of the foodstuffs existed.

Per 100 grms. dry matter (nitrogen expressed throughout as protein)

	Bag 1		Bag 2		Bag 3		Bag 4	
	Green	Silage	Green	Silage	Green	Silage	Green	Silage
	%	%	%	%	%	%	%	%
Amino acids, etc.	0.11	4.76	0.11	4.38	0.11	3.61	0.08	4.24
Volatile bases	0.02	1.17	0.02	1.09	0.02	1.05	0.03	0.85
True amides	2.82	1.31	2.82	1.38	2.82	1.19	2.37	0.97

	Bag 5		Bag 6		Bag 7		Bag 8		
	Green	Silage	Green	Silage	Green	Silage	Green	Silage	
	crop		crop		crop		crop	Unspoilt	Spoilt
	%	%	%	%	%	%	%	%	%
Amino acids, etc.	0.08	4.74	0.05	4.13	0.06	2.81	0.06	2.95	1.92
Volatile bases	0.03	0.87	0.02	0.88	0.03	0.82	0.03	1.08	1.55
True amides	2.37	0.55	2.43	1.52	3.54	2.25	3.54	1.46	0.35

The amino acids (with amides of the asparagine type) and volatile bases are determined by titration of the extracts. The result obtained by subtracting the sum of these from the "amides" as determined in the analysis of the dry matter of the samples has been designated "true amides"; *i.e.* amides which do not contain a free carboxyl group and thus escape determination during titration of the extracts.

It is of interest to note that amino acids and amides like asparagine comprise only a very small fraction of the "amide" constituent of green oats and tares. This fact is in harmony with the conception that these substances represent stages in the synthesis of plant protein and therefore exhibit no tendency to accumulate in the plant.

The outstanding features of the silage "amide" figures are: 1. A big increase in the amount of amino acids, these forming, in the case of unspoilt samples, the bulk of the "amides" of the silage. 2. An increase in the amount of volatile bases. 3. A decrease in the amount of "true amides." The increase in the volatile bases is probably explained by the production of ammonia by a hydrolytic change affecting the "true amides." Thus, in bag 1, 1.5 per cent. "true amides" disappeared and 1.15 per cent. volatile bases made their appearance. The figures for bag 8, where spoiling occurred, are exceptional. In the case of the spoilt portion of bag 8 silage, the amounts of amino acids and "true amides" were low, whereas the amount of volatile bases was relatively high.

Such features may be regarded as the chemical evidence of spoiling, the volatile bases having made their appearance as a result of the destruction of amino acids. In the production of good silage, the main changes affecting the nitrogenous constituents are probably brought about by proteolytic enzymes and an investigation of the material reveals a high ratio of amino acids to volatile bases. The rotting of silage is evidenced by a low ratio. The ratio in the case of bag 7 silage affords evidence of the slight degree of spoiling which actually did occur in this bag. A further illustration is seen in the figures already given for the titration of the extract of the spoilt silage sample from the top of silo 11. Here the volatile bases are actually present in excess of the amino acids, the ratio of amino acids to bases being roughly 1 : 1.6.

In bags 1, 2 and 3, where large quantities of juice were lost by drainage, there were appreciable losses of crude protein, probably in the form of "amides" dissolved in the juice. In bags 4, 5, 6 and 7, where little or no juice was lost by drainage, only small changes in the crude protein constituent were recorded. The slight gain of protein in bags 4 and 5 arose probably from experimental error (difficulty of accurate sampling, etc.). It may safely be assumed that if silage can be made without excessive drainage from the silo, then the loss of crude protein will be small. A twofold problem awaits satisfactory solution if the practice of ensilage is to develop on a sound economical basis: 1. The prevention of excessive drainage from the silo. 2. The possible utilisation of silage juice in feeding. Both these questions are receiving attention at the present time.

In every case the crude protein digestibility of the green oats and tares (as determined *in vitro*) suffered a slight depression as a result of ensilage. A study of the data points to the probability that the depression of protein digestibility is greatest where large losses of the easily assimilated "amides" occur as a result of drainage. Where spoiling occurs, as in bag 8, the decrease in protein digestibility may be considerable.

III. *Changes suffered by the Ether Extract.*

In all the bags, with the exception of bags 7 and 8, the amount of ether soluble material was augmented as a result of the changes undergone by the green forage during ensilage. The increase was very variable in amount and apparently bore no relation to the percentage loss of nitrogen-free extractives. Thus, in bag 4, where about 12 per cent. of the nitrogen-free extractives disappeared, there was a 13 per cent.

increase in the ether extract, whereas in bag 6, an almost equal percentage destruction of nitrogen-free extractives was accompanied by a 35 per cent. increase in the amount of ether extract.

The results obtained in this regard for bags 1 and 2 were curious. In both bags the percentage loss of nitrogen-free extractives was about 19 per cent.; in bag 1, however, the increase of ether extract was 23 per cent., the corresponding figure for bag 2 being only about 9 per cent. As bag 2 occupied the upper half of the small silo, it is possible that a portion of the volatile organic acids escaped as a result of "heating," since analysis of the extracts showed that the bag 2 silage was much poorer in respect of volatile acids than was the bag 1 silage.

In bags 7 and 8, where spoiling occurred, there was an appreciable *decrease* in the amount of ether extract during ensilage. It follows that where silage undergoes rotting, the process is attended by losses of ether soluble material. It has already been demonstrated that the organic acids are readily destroyed during the spoiling of silage by moulds.

If the figures for the ether extracts be compared with those obtained in the titration of the organic acids in the extracts, certain interesting facts are disclosed which merit further investigation. The figures obtained for the green crop extracts show that the green oats and tares contained an appreciable amount of non-volatile material which titrated with $N/10$ NaOH in the presence of phenolphthalein. The amount of volatile acidic constituent was negligible. The nature of the non-volatile acidic constituent was not ascertained. It was observed, however, that during titration of the green crop extracts, a strong yellow colour developed, which intensified as the neutral point was approached. It is probable that the oats and tares contain a constituent which reacts with the soda producing the colour change, and the soda used up in this reaction accounts for the high non-volatile acidic figure obtained for the green crop. As this phenomenon was noticed in an equal degree during the titration of the silage extracts, it follows that this constituent escapes destruction during ensilage, and thus it is not feasible to calculate the whole titration figure for non-volatile acidity in the silage in terms of lactic acid. It was further noted that the spoiling of silage by moulds occasioned the destruction of the constituent in question.

The following table gives a comparison of the percentages of ether extract in the *dried* silage samples with the non-volatile acidity calculated as lactic acid, after subtracting from the non-volatile acid titration figure the corresponding figure for the green oats and tares.

Per 100 grms. dry oat and tare silage:

Bags	1	2	3	4	5	6	7	8 Unspoilt sample
Non-volatile acidity calculated as lactic acid, %	6.53	7.22	6.11	3.11	2.51	5.17	2.85	2.48
Ether extract in dry matter, %	3.12	3.21	3.37	3.18	3.15	3.05	2.81	2.70

It would be anticipated that the amount of ether extract in the silage after drying at 100° would show some correspondence with the amount of non-volatile acidity calculated as lactic acid. This was the case in the material of bags 4, 5 and 7 and in the unspoilt portions of bag 8. Wide discrepancies occur, however, in the figures for the silage in bags 1, 2, 3 and 6, warranting the assumption that a very different acidic fermentation has taken place in these bags from that which occurred in the remaining bags. It is clearly impossible to assume that the non-volatile acid in these bags was lactic acid wholly. In this connection, it is interesting to remember that bags 1, 2 and 3 contained unwilted material, and bag 6 slightly wilted material, and that the fermentative processes in these bags went on therefore under much moister conditions than obtained in the other bags. The question is worthy of further investigation, since excellent samples of silage were obtained from bags 1, 2 and 6. It is probably incorrect to assume that lactic acid is the only non-volatile organic acid which may arise as a result of fermentative changes in the silo.

IV. *Changes suffered by the crude fibre constituent.*

In every case, a loss of crude fibre was recorded as a result of ensilage, the loss varying from about 5 per cent. in the case of the unwilted oats and tares to about 10 per cent. in the case of the wilted material. In the case of the spoilt sample in bag 8, the loss of crude fibre was as much as 16.5 per cent. of the original amount of fibrous constituent in the bag.

The question arises as to the type of action in the silage process which results in the disappearance of crude fibre. The explanation is probably to be found in work carried out by Voelcker¹, who found that the following change occurs when straw chaff is mixed with a small quantity of green rye or tares (1 ton straw chaff to about 1 cwt. of rye or tares) in the spring or summer and the mixture is allowed to ferment until the autumn.

The untreated straw contained 23.7 per cent. N.-free extractives and 54 per cent. crude fibre.

The fermented straw contained 45.8 per cent. N.-free extractives and 34.5 per cent. crude fibre.

¹ Voelcker, *J. of the R. Agric. Soc. of Eng.* **7**, 85, 1871.

It is thus obvious that the fermentation to which the straw was submitted had the effect of converting an appreciable amount of the cellulose of the straw into a form which could be dissolved during the process of determining the percentage of crude fibre. The conversion of crude fibre into nitrogen-free extractives involved a distinct improvement in the feeding value of the straw.

From the results of the present investigation, it appears probable that the cellulose of the green oats and tares undergoes to some extent a similar breakdown during ensilage, resulting in a gain of nitrogen-free extractives and a corresponding decrease in the amount of crude fibre. Indeed, the nitrogen-free extractives so formed may conceivably undergo further change with the production of organic acids, although this may not happen if the green forage develops acidity rapidly in the early stages of storage in the silo.

It should be pointed out that the oats and tares used in this investigation were cut in an immature condition, so that the optimum conditions obtained for observing possible changes affecting the fibrous constituent. To what extent the cellulose of mature forage would be subject to such changes is uncertain, though Voelcker's work indicates that similar changes would occur. The point is under investigation.

The changes modifying the cellulose constituent during ensilage of fodder are of great importance from the point of view of nutritive value. The gain is twofold: 1. Part of the fibre breaks down into nitrogen-free extractives (and, to some extent possibly, to organic acids). 2. The residual crude fibre itself possesses a greater digestibility than the original crude fibre in the green oats and tares¹.

V. Changes suffered by the nitrogen-free extractives.

In no case was the loss of nitrogen-free extractives less than 11 per cent. of this constituent originally present in the bags. The biggest losses were registered in bags 1 and 2, the diminution being roughly 19.5 per cent. in each bag. It is significant, in view of the remarks contained in the preceding paragraph, that the amount of crude fibre disappearing in these bags was much smaller than in the remaining bags.

The results for bag 8 show that the rotting of a silage sample may lead to large losses of nitrogen-free extractives.

VI. Changes suffered by inorganic constituents.

Large losses of inorganic salts may occur during ensilage owing to the soluble portions escaping in the juice. Where little drainage occurs,

¹ Woodman *J. Agric. Sci.* **12**, 144, 1922.

the loss of these constituents is not appreciable. These facts must be considered in conjunction with the importance attaching to the inorganic salt content of a foodstuff from the nutritional standpoint. If, for example, silage is to be utilised in feeding dairy cows, it is undesirable that the green crop should sustain large losses of inorganic salts during ensilage.

SUMMARY.

Experiments have been described which had primarily as their object the investigation of the effect of varying moisture content in the green oat and tare crop on the type of silage produced from such forage. The magnitude of the changes affecting the constituents of the green crop under the different conditions of ensilage have also been detailed.

The main conclusions are summarized below:

(1) The ensiling of a fresh green crop in an early stage of maturity (soon after flowering) with little or no previous wilting, and with a fermentation temperature in the neighbourhood of 25°C ., leads to conditions which favour the production of green "fruity" silage. The results of the experiments suggest that with this type, a considerable loss of dry matter may result from the process, especially if much drainage of sap occurs. The same conditions appear to be favourable to the extensive splitting up of true protein into soluble nitrogenous products; more than 50 per cent. of the true protein of the green crop may be transformed into "amides," an appreciable proportion of which may be lost, together with inorganic salts, in the drainage juice.

Thus, though green "fruity" silage is much relished by stock and possesses excellent feeding value, yet its production may be accompanied by substantial losses of crude protein and soluble salts. Loss by drainage should therefore be obviated.

(2) The ensiling of a moderately dry crop, dried either by wilting or by allowing the crop to mature, produces conditions which favour the production of the acid brown type of silage. The production of this type of silage is accompanied by a relatively low loss of dry matter, the amount of juice drainage from the silo being very much smaller than that occurring during the production of green "fruity" silage. Approximately 30 per cent. of the true protein of the green crop is split up into "amides."

(3) The ensiling of material which has undergone prolonged wilting and extensive rain-washing does not produce a good quality of silage, and the forage displays a tendency to become mouldy during the process.

Where moulding occurs, the volatile and non-volatile organic acids are not simply neutralised by basic products, but are actually destroyed. The process is accompanied by large losses of dry matter, the "amides," nitrogen-free extractives and ether extract being extensively destroyed. Chemical evidence of the spoiling of silage by mould development is afforded by a study of the ratio of amino acids to volatile bases in the silage extract. In good silage, the ratio is high; in spoilt silage, the volatile bases may be present actually in excess of the amino acids.

(4) In all the samples of normal silage investigated, the non-volatile organic acids were present in good excess of the volatile organic acids. Evidence has been brought forward warranting the assumption that the acidic fermentation during the formation of green "fruity" silage is markedly different from that accompanying the production of brown acidic silage, and it appears probable that lactic acid is not the only non-volatile organic acid which may arise as a result of the fermentative action in the silo.

(5) The increases in the amount of ether extractable material as a result of ensilage are very variable in the different experiments and bear no relation to the percentage losses of nitrogen-free extractives.

(6) In every case, the crude protein digestibility of the green oats and tares (as determined *in vitro*) has been shown to suffer a slight depression during ensilage.

(7) The outstanding features of the silage "amide" figures as compared with the corresponding figures for the green crop are: (a) a large increase in the amount of amino-acids, these forming the bulk of the "amides" of the silage; (b) an increase in the amount of volatile bases, the latter consisting probably of ammonia which has arisen as a result of hydrolytic changes affecting amides originally present in the green crop.

(8) Results have been obtained which suggest that the cellulose of green oats and tares undergoes to some extent a breakdown during ensilage, resulting in a gain of nitrogen-free extractives and a corresponding decrease in the amount of crude fibre. Furthermore, as has been demonstrated in a previous communication, the crude fibre remaining in the silage possesses a greater digestibility than that originally present in the green forage.

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THE AVAILABILITY OF MINERAL PLANT FOOD.

A MODIFICATION OF THE PRESENT HYPOTHESIS.

By NORMAN M. COMBER.

(*Department of Agriculture, The University, Leeds.*)

THE PRESENT HYPOTHESIS.

SCIENTIFIC soil researches have passed through one fairly well defined phase. The important and pioneer work of empirical field trial has established the main physical and chemical requirements of crops and laid down the routine method of examining soil amendments.

The researches of recent years have inaugurated a second phase: an enquiry into the constitution of the soil and into the mechanism of the growth of plants in soil. In connection with this second phase of soil investigations much progress has been made on the botanical side in developing knowledge of the mechanism of processes which go on inside the plant, and in the last decade views of the constitution of the soil have changed fundamentally. But the relation of the plant to the soil has received very scanty consideration in modern literature. Views on that subject are very much as they were after Daubeny's publication in 1845. Outstanding work has been done by Dyer¹, by Cameron and Whitney², and by Hall³ and his collaborators. There have been some changes of interpretation and some differences of opinion, but the fundamental assumption that the soil solution is the nutrient medium of the plant, that the state of solution is necessary to availability has remained almost unquestioned.

The basis of practically all teaching on the chemistry of soil fertility and on the value of fertilizers, is the belief that nutrient substances pass into solution in the soil water *externally to the plant* and subsequently diffuse into the root hairs. Much well-known discussion has arisen about the solvents concerned, particularly about the possible excretion of organic acids by the plant.

¹ *Trans. Chem. Soc.* 1894, 65.

² *U.S. Dept. Agric. Bureau of Soils Bull.* 1903, No. 22.

³ *Phil. Trans.* 1913.

THE INADEQUACY OF THE PRESENT HYPOTHESIS.

There are a number of facts which are difficult to reconcile with this hypothesis.

1. *The relation of the composition of the soil solution to the mineral elements taken up, and the water transpired, by plants.* In obvious and natural accordance with the view that the soil solution is the nutrient solution of the plant, many attempts have been made to express or displace this solution from the soil, and by its analysis to ascertain the essential chemical knowledge of the fertility of the soil concerned. The results of these experiments show that the ratio of certain mineral elements assimilated by plants to the water transpired is greater than the ratio of these elements to the water in which they are dissolved in the soil. Hall¹ has calculated that if the potash taken up by a clover crop is assumed to have been dissolved in the water transpired, the calculated solution is far more concentrated in respect of potash than solutions obtained from the soil. Ramann² makes a similar observation but arrives at a different conclusion. Hall deduces that the dissolved material enters the plant from the soil solution at a greater rate than the water. Ramann is apparently unable to reconcile this view with the laws of diffusion and concludes that the plant takes material from the soil other than that which is in solution.

2. *The absorption of iron by plants.* Iron is a necessary element for plant growth. All plants take up a certain amount from the soil. The amount necessary is relatively small, but it is difficult to see how even this small amount can exist *in solution* in soils containing a high percentage of chalk.

3. *The availability of phosphates.* This is probably the greatest difficulty of all. The value of various phosphatic substances to plants is not by any means in accordance with the present hypothesis. Basic slag and certain mineral phosphates are sparingly soluble and are yet valuable phosphatic fertilizers. The evaluation of these substances entirely on a solubility basis has not been an unqualified success. Their usefulness as phosphatic fertilizers is often comparable to that of a water soluble phosphate.

The phosphates of iron and aluminium are in a curious position in the literature. On the one hand it is normally taught that these are insoluble

¹ *The Soil*, 1920.

² *Landw. Ver.Stat.*, 1916, 88.

and of little use to the plant, and that the formation of these phosphates in superphosphates diminishes the value of the material to the plant. On the other hand, direct experiment¹ leaves no doubt that phosphates of iron and aluminium are very useful to the plant as sources of phosphorus. There seems to be a prejudice, which prevails in spite of experimental evidence to the contrary, that sparing solubility must mean a low availability. The experimental fact is that sparingly soluble phosphates are often easily available, and the inevitable conclusion is that solubility is not the dominating condition of availability.

A MODIFICATION OF THE PRESENT HYPOTHESIS.

The hypothesis that all material entering the plant from the soil first becomes dissolved in the soil water and then diffuses into the root hair, arose on the basis of two fundamental beliefs. First, the physiological belief that only material in true solution can diffuse through the cell protoplasm and second, the belief that the soil is a simple system of particles moistened by a solution. Now these two fundamental conceptions have become modified. Because of the modifications and also because of the discrepancies already noted between the facts and the hypothesis, it is desirable that the hypothesis should be reconsidered.

The absorption of colloids by the plant. As a first point in the reconsideration it may be noted that the teaching that only material in true solution can diffuse into the plant cells is not unquestionably true. There is some evidence that less highly dispersed material can enter. Czapek² definitely states that colloids can enter the plant cells. "Die lebende Plasmahaut ist nicht nur für echte Lösungen, sondern auch für kolloide Lösungen durchlassig." He refers to the passage of dyestuffs and of fat emulsions into plant cells. Pfeffer³ records the diffusion of silicic acid into the cell sap of plants.

There is also some recent experimental evidence in favour of the absorption of colloidal silica by plants. Jennings⁴ describes a series of experiments in which wheat seedlings were grown in water solutions to which were added various absorbing substances. When colloidal silica gel was used there was a marked increase in growth and in dry matter, and it was clearly shown that the plant had absorbed silica.

¹ See Marais, *Soil Sci.* 1922, **13**, No. 5 and bibliography.

² *Biochemie der Pflanzen*, 1913.

³ *The Physiology of Plants*, 1900.

⁴ *Soil Sci.* 1919, **7**.

The following are some of Jennings' figures:

Dry weights and silica contents of wheat seedlings grown in nutrient solutions and in nutrient solutions containing silica (Jennings).

				Dry weight of tops	Silica in dry matter
				gm.	%
Nutrient solution 85 pts per million			(without silica	0.2628	5.3
			(+ silica gel 1 %	0.4669	16.0
..	250	..	(without silica	0.5635	3.1
			(+ silica gel 1 %	1.0510	20.2
..	500	..	(without silica	0.5743	2.8
			(+ silica gel 1 %	1.1404	22.8
..	1000	..	(without silica	0.9688	2.0
			(+ silica gel 1 %	1.4235	31.3

Thus it appears that colloidal gels can enter the plant, and entering the plant from the soil they may carry with them absorbed substances.

The essential elements for plant growth. It is commonly held that there are ten essential elements involved in the growth of plants. That tenet implies that all other elements universally found in plants grown in soil are there by accident. That this teaching has not been thoroughly acceptable is well known and from a study of plants grown in soil it has been contended that silicon and a number of other elements are essential. The argument for ten essential elements and no more is, of course, the fact that apparently normal plants can be grown in water solutions without the introduction of any detectable trace of elements other than the well-known ten. That fact proves that only ten elements are required for the growth of plants *in water solution*. The application of the same conclusion to plant growth *in soil* is entirely dependent upon the assumption that the mechanism of feeding is the same in the soil as in water solution. If the mechanism of feeding is different the chemical requirements may well be different too. If, for instance, silica acts as a kind of carrier of mineral substances to plants grown in soil, silicon will be an essential element for that purpose. By considering the possibility of a mode of absorption from soil different from that which obtains in the case of plants grown in water solution, some explanation of the additional elements always present in soil-grown plants may be found.

The relation of the root hairs to the soil particles. Another point arises from the modified view of the constitution of the soil. Whereas the soil system was formerly regarded as merely a system of moistened particles it is now regarded as a system of particles which are connected with the external and free water by gel material. Some attention has been given to the bearing of this conception of the soil on transpiration and the

water content of plants¹. Its bearing on the mineral nutrition of plants still awaits consideration.

Now it is a well-known botanical fact that when the root hair comes into contact with a soil particle the outer layer of the root hair is transformed into a mucilage and an indissoluble attachment is made with the soil particle. One relation of the soil colloids to the plant is here apparent. The soil particle is coated with hydrophilous colloid. The root hair becomes coated with hydrophilous colloid. By the union of these colloids the plant and the soil particles become cemented together. The particles so attached to the plant cannot be removed without damage to the root hairs.

Underlying the present teaching is the conception of the root hair "dipping into" the soil solution, and taking up its nutrient material in exactly the same way as it does from experimental water culture solutions. It seems, however, important to acknowledge the fact that *by the union of their respective colloids the plant and the soil form one system and not two systems in mere contact*, and to admit the possibility that the mechanism of the nutrition of plants grown in soil is not necessarily and entirely the same as the mechanism of the nutrition of plants grown in water solution.

The physical possibilities of the union of the plant and the soil. The migration of ions in the colloidal complex by which the plant and soil are united has been discussed by Casale². He argues that positive and negative colloids exist in the soil and that the charge on them is due to their throwing off anions and cations respectively. The negative colloids are dominant and absorb the positive colloids. The colloids of the plant, according to Casale, throw off hydrogen ions and are negatively charged, but less so than the soil colloids. Hence there is a potential difference between soil and plant and a migration of cations from the soil to the plant accordingly. Equilibrium is never reached, because the cations pass on into the plant by a similar electrical mechanism.

Sufficient seems to be known about the different electrical charges on various parts of the plant cells to indicate that a migration of ions under difference of electrical potential probably plays an important part in plant nutrition and cannot be left out of account in a consideration of the availability problem.

The chemical possibilities of the union of the plant and the soil. When the root hair and the soil particle are cemented together there is an obvious possibility of the cell sap of the root hair dissolving material

¹ See Shull, *Trans. Faraday Soc.* 1922, **17**.

² *Stuz. sper. agr. ital.* 1921, **54**; *J.C.S. Abs.* (i), 1922, 509.

from the soil aggregate without any excretion of acid into the soil generally. The cell sap and the soil particles to which the cell is cemented are in such contact as to admit the direct dissolution from particular particles by the sap of the particular cells attached thereto.

Organic acids have a far greater solvent power for many mineral substances than mineral acids have. Oxalic acid will dissolve much larger amounts of iron from the soil than hydrochloric acid. Calcium phosphate is much more soluble in citric, acetic, lactic and malic acids than in hydrochloric or nitric acids. But it must be noticed that the solution of mineral matter by the sap and mucilage of plant cells is not to be regarded as essentially an *acid* dissolution. Many organic compounds besides acids will dissolve iron and aluminium compounds and phosphates. Tricalcic phosphate, for example, is much more soluble in water containing starch, glue, sugars and many other organic bodies.

This effect of organic matter on the solubility of phosphates and of compounds of iron and aluminium is very well known and in soil phenomena it must be far reaching. It affords a satisfactory explanation of the uptake of iron from chalky soils, for many organic compounds have a high solvent power for iron over a wide range of both acid and alkaline reaction. The enormous amount of oxalate in lichens growing on limestone is not without significance in this connection.

The availability of sparingly soluble phosphates becomes more intelligible if it is supposed that the root hairs become cemented to the particles thus admitting the absorption of colloidal phosphate and the *direct* dissolution of the particle by the organic solvents concerned. Also it is easy to see the connection between the root habit of wild white clover and its special response to the presence of these sparingly soluble phosphates.

The available mineral phosphates have, according to the argument of a succeeding paper¹, a hydrophilous surface with which the root hair can make its attachment. The unavailable phosphates appear not to have a colloidal surface. Ferric phosphate loses its colloidal properties on ignition and it also, according to Prianischnikow² becomes unavailable for the plant. Aluminium phosphate retains its colloidal properties after ignition and it remains available for the plant.

There are, therefore, strong indications that colloidal properties in sparingly soluble mineral plant food are of first importance, perhaps enabling the plant to absorb colloidal matter; certainly enabling the

¹ This Volume, p. 372.

² *Biol. Centr.* 1905, **34**.

root hair to make its natural attachment. The importance of such properties has already received some recognition commercially: a process has recently been patented¹ whereby "insoluble phosphates...are converted to a colloidal form and rendered suitable for use as manures by treatment with a large quantity of water and about 0.1 to 0.3 per cent. of a mineral acid or alkali in a high speed disintegrator...substances which act as protective colloids, e.g. tannin, or salts of lysalbinic acid or humic acid, or the like, may also be added."

According to the differences in the composition of the cell sap and of the mucilage developed by the root hair, so plants will vary in their power of attachment to mineral particles and in their power to dissolve material from the particles to which they are attached. There is no measure of availability apart from the plants concerned and the conditions of their growth.

SUMMARY.

The assumption that plants feed in the soil just as they feed in water culture solution is unjustified and contrary to the facts. In modification of the usual hypothesis two possibilities are discussed.

1. The absorption of colloids by the plant.
2. The union of the root hair with soil and other mineral particles (so that the plant and the soil form one system) and the dissolution of the particle by the organic matter of the root hair so attached.

¹ See *J. Soc. Chem. Ind.* 1922, **41**, No. 10, 385 a.

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A MODIFIED TEST FOR SOUR SOILS.

BY NORMAN M. COMBER.

(*Department of Agriculture, The University, Leeds.*)

It was recently suggested¹ by the writer that the neutral salt test for sourness in soils could be adapted for rapid qualitative purposes by using an alcoholic solution of potassium thiocyanate. This reagent becomes coloured in contact with soils deficient in strong bases, because of the presence of iron among the weak bases which are brought into solution from such soils by neutral salts.

This thiocyanate test appears to have been used quite extensively in this country, in Denmark and Scandinavia; and one attempt² has been made in America to make it a quantitative lime-requirement method.

The use of this test by farmers and others not in close contact with a chemical laboratory is very restricted on account of the difficulty of obtaining either the alcohol or the thiocyanate. Also, the reagent is expensive and highly poisonous. A minor inconvenience is the necessity of drying the soil: freshly sampled soils often contain enough water to destroy the colour.

Some less restricted modification of the test was therefore sought, and it was found that *an aqueous solution of potassium salicylate* is a useful substitute for an alcoholic solution of the thiocyanate.

In the absence of large amounts of mineral acids, salicylic acid and its salts give a violet colour with traces of ferric salts. In aqueous solution this test for iron is far more delicate than the thiocyanate test. Aqueous solutions of ferric salts are found to give the violet colour with salicylic acid in dilutions too great to admit of coloration by thiocyanate.

When, therefore, a solution of potassium salicylate is applied to a soil which yields iron to neutral salt solutions, the appearance of this violet colour in the solution might be expected. Accordingly the action of this reagent was examined in the first instance on twelve soils known to be sour and to respond to the thiocyanate test, and on twelve soils

¹ This *Journal*, 1920, **10**.

² Carr, *Journ. Ind. Eng. Chem.*, 1921, **13**, No. 10.

known not to be sour. A *red* colour developed in a few minutes in the solutions in contact with the sour soils and a yellow or brownish-yellow colour in the others. This brownish-yellow colour is apparently developed in all cases, and the red colour arising from the sour soils is due to the combination of the brownish-yellow and violet colours.

During the last nine months the test has been applied to a large number of soils and its indications agree with those of the thiocyanate test.

The colour developed by aqueous potassium salicylate is seldom so deep as that developed by alcoholic thiocyanate, and the soil takes longer to settle from the water than from the alcohol.

A 5 per cent. solution of salicylate is one of convenient concentration, but more concentrated solutions give a quicker indication.

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THE FLOCCULATION OF SOILS. III.

By NORMAN M. COMBER.

(*Department of Agriculture, The University, Leeds.*)

IN the previous papers¹ it has been deduced that the flocculation of soil particles by calcium hydroxide is the net result of its deflocculating action on the cores of the particles and its precipitating action on the colloidal matter. Calcium hydroxide will deflocculate or flocculate according to which of these actions is dominant.

In the present communication further support of the earlier deductions is offered from experimental observations on

- (i) The flocculation of particles other than soil particles.
- (ii) The effect of colloidal silica on the suspensibility of particles.
- (iii) The effect of concentration on the relative flocculating powers of calcium hydroxide and calcium chloride.
- (iv) The relative lime absorbing capacities of the core and of the colloidal surfaces of soil particles.
- (v) The effect of heat on soils.

EXPERIMENTAL.

A. *The flocculation of particles other than soil particles.*

1. Experiments such as those described in the first of these papers were made with a variety of relatively insoluble powders, in order to ascertain whether any substances other than clay were precipitated from their suspensions better by calcium hydroxide than by a neutral calcium salt. It was difficult to get very durable suspensions of many of these powders. However, such examination as could be made indicated that the abnormal flocculation by calcium hydroxide was *not shown* in suspensions of zinc oxide, zinc carbonate, ferric oxide, barium sulphate, ferric phosphate (ignited), lead carbonate and Canadian apatite. It *was shown* in suspensions of aluminium phosphate (unignited or ignited),

¹ *Journ. Agric. Sci.* 1920, **10** (4); 1921, **11** (4).

ferric phosphate (unignited), basic slags (after the removal of free base), finely powdered raw bones, and rock phosphates¹ (after removal of free lime).

2. Aluminium phosphate suspensions were subjected to further examination. With three different samples, two purchased and one prepared in the laboratory, most of the experiments were carried out that have been described in the two previous papers in connection with clay suspensions. The aluminium phosphate behaved in many respects like clay. It was flocculated by calcium compounds better in alkaline than in neutral suspensions; the use of ammonium hydroxide in conjunction with a calcium salt produced a much larger volume of coagulum than when the calcium salt was used alone; at very low concentrations calcium hydroxide was inferior to calcium chloride as a flocculant, and a concentration of ferric chloride could be found which gave an optimum flocculation.

B. The effect of colloidal silica on the suspensibility of small particles.

Reference has been made in the earlier papers to the effect of very small amounts of colloidal silica on the flocculation of suspensions of ferric oxide.

The effect of silica on the suspensibility of particles has been frequently noticed throughout these experiments. Specific observations were made by weighing out 0.25 gm. portions of the powder concerned, suspending one portion in 10 c.c. distilled water and the other in 10 c.c. of a silica sol containing 0.0031 gm. SiO_2 in the 10 c.c.

The silica increased in a marked manner the suspensibility of two different samples of ferric oxide, zinc carbonate, ignited soil particles, kaolins and other lean clays. With other substances, e.g. zinc phosphate, powdered granite, powdered felspar, no effect was visible.

C. The effect of concentration on the flocculating power of calcium hydroxide.

In the previous paper an experiment was described which shows that in very low concentrations, calcium hydroxide has a flocculating power less than that of calcium chloride, while at higher concentrations the reverse is true. Similar experiments with other clays and with kaolin have shown a similar result. Two of these experiments will be quoted.

1. A London Clay subsoil was extracted with 5 per cent. HCl, and thoroughly washed. One portion of the extracted subsoil was shaken

¹ Nine mineral phosphates were kindly supplied by Dr G. Scott Robertson.

with water and allowed to settle. The clay was then decanted. The remaining portion was treated with excess of lime water and left for one day with frequent shaking. This portion was then filtered and washed with hot water until the washings gave no colour with phenolphthalein. It was then shaken with water and allowed to settle after which the clay was decanted. Two suspensions of the clay were thus obtained, one depleted of its easily soluble bases and the other with as much lime as it could retain against a hot water washing. These suspensions were then adjusted so that 100 c.c. of each contained 0.2270 gm. clay (weighed after ignition). The relative effect of Ca(OH)_2 and CaCl_2 in various concentrations was then observed on 10 c.c. portions of each of these suspensions. It was found that the suspensions of the acid extracted clay were flocculated better by the Ca(OH)_2 at all concentrations above about $N/150$, while the suspension of clay after treatment with Ca(OH)_2 was flocculated better by Ca(OH)_2 at all concentrations above $N/1000$.

The amount of lime already held by the soil clearly affects the concentration of added Ca(OH)_2 above which the abnormal effect of that hydroxide is manifested.

It must also be remarked that the clay which had been treated with lime water and washed remained suspended in the control tubes very much longer than the clay which had been extracted by acid and washed.

2. A similar comparison of the flocculating powers of Ca(OH)_2 and CaCl_2 was made with two different clays, one a fat Halifax clay and the other a lean kaolin. The suspensions used contained 0.3014 gm. ignited clay in 100 c.c. In the experiment with the fat clay the superior flocculating power of Ca(OH)_2 was operative and *very pronounced* at all concentrations above $N/500$. In the experiment with the kaolin the superior flocculating power of Ca(OH)_2 could be seen at all concentrations above $N/1000$; in the course of one or two minutes, however, the neutral and alkaline suspensions were alike. The phenomenon could be seen with the lean clay but was *not very pronounced*.

Similar experiments with other clays showed that the superior flocculating power of Ca(OH)_2 at higher concentrations was always more pronounced in suspensions of fat clays than in suspensions of lean clays.

D. *The relative lime absorbing capacities of the core and of the colloidal surface of soil particles.*

1. When a soil has been extracted with acid, its base absorbing power and its power to decompose calcium carbonate are usually in-

creased. Experiments made by Schollenberger¹ indicate, however, that there is a limit to the increase in base absorbing power which is brought about by acid extraction. Working with a Clyde clay he found the base absorbing power increased with the concentration of the acid used up to an acid concentration of about $N/2.5$, but greater concentrations of acid failed to produce any further increase in the base absorbing power. With a Miami clay loam Schollenberger found that extraction with $N/10$ acid brought the soil to its maximum base absorbing power.

The strong indication of these results is that the base absorbing power of soils as measured by the ordinary "lime requirement" methods, is a phenomenon exclusively confined to the colloidal surface of soil particles and is not to any appreciable extent a simple and direct reaction with the structural minerals forming the cores of the particles, as is suggested by Sullivan² and others.

In further examination of the point, seven soils were taken at random and samples of each, after passing the 1 mm. sieve were extracted for one day under similar conditions with HCl of various concentrations. 35 gm. soil and 100 c.c. acid were used for each extraction. The soils were filtered, washed, and air dried. The "lime requirement" was determined by the Hutchinson-McLennan method. The figures are as follows and fully confirm Schollenberger's results.

Table I.

"Lime Requirements" of soils after extraction with HCl of various concentrations.

(CaCO_3 per 100 of Soil.)

Concentration of HCl	Soil						
	Medium subsoil (Chelmsford)	Heavy soil (Batley)	Light soil (Garforth)	Millstone grit soil (Leeds)	Millstone grit soil (Sheffield)	Millstone grit soil (Shipley)	Millstone grit soil (Knaresborough)
$N/100$	Nil	0.74	—	—	—	—	—
$N/50$	0.08	1.04	0.23	—	0.39	—	—
$N/10$	0.55	1.10	0.33	0.32	0.73	0.54	0.41
$N/4$	0.54	—	0.33	0.32	0.75	0.55	0.43
$N/3$	—	1.10	0.33	—	—	0.55	—
N	0.54	1.10	0.30	0.33	0.75	0.55	0.43
$2N$	0.53	—	—	—	0.75	—	—
$10N$	0.50	1.04	0.27	—	—	—	—

2. Attempts were made to carry out similar experiments with orthoclase feldspar and with powdered granite, but the base absorbing powers of these after acid extraction were so small that the attempt was

¹ *Soil Science*, 1917, 3.

² *U.S. Geol. Survey Bull.*, 1907, 312.

abandoned. After extraction with *N*/3 acid the felspar showed a "lime requirement" of 0.01 per cent. CaCO_3 and the granite showed no "lime requirement" measurable by the Hutchinson-McLennan method.

E. *The effect of heat on soil aggregates.*

It has been contended in these papers that the aggregation of soil particles is brought about and maintained by the binding or cementing action of gel material. If this is true, one of the first effects of heating a soil will be the dehydration, shrinkage and cracking of the gels and presumably a loosening of the aggregate and a consequent increase in the exposed surface.

It is well known that heating a soil under certain conditions increases the soluble matter¹. The experiments recorded in this section have been made in order to find evidence for or against the view that this increase is in part due to the increased surface exposed by the destruction of the cementing gels.

1. (a) It has been claimed by Fraps² that after the ignition of a soil for a few minutes over a bunsen flame the amounts of iron, aluminium and phosphorus soluble in dilute HCl are increased. Repetitions of these experiments with a number of English soils abundantly confirm Fraps' results. For example, the following figures show the weights of Fe_2O_3 and Al_2O_3 extracted from 100 gm. London Clay subsoil by 250 c.c. *N*/5 HCl before and after 5 minutes' ignition.

	Unignited	Ignited
Fe_2O_3	26 mgm.	186 mgm.
Al_2O_3	174 ..	530 ..

(b) In order to test qualitatively the effect of the partial ignition of soils and other systems, on the solubility of iron in dilute acid, equal amounts of the material under examination were weighed out, and one portion ignited over a bunsen flame for a few minutes. Each portion was then extracted with the same volume of *N*/5 HCl for 15 minutes and filtered. 1 c.c. of a standard solution of NH_4CNS was added to equal volumes of the filtrates and the colours compared. In this way an increase in the solubility of iron in dilute acid was shown to be brought about by the partial ignition of a large number of soils, subsoils and clays, and of some synthetic systems to be presently described.

(c) Experiments similar to the foregoing were made using $\text{K}_3\text{FeC}_6\text{N}_6$ as the reagent, and it was found that a considerable amount of ferrous iron in the acid solution resulted from partial ignition.

¹ For a review of the literature see Gustapon, *Soil Science*, 1922, **13**.

² *Journ. Ind. Engin. Chem.* 1911, **3**; *J.C.S. Abstracts*, ii. 1912.

(d) It is well known that ignition ultimately depresses the solubility of iron and other soil constituents. Clearly therefore the rise in solubility—or rather in the amount dissolved—takes place only in the early stages of ignition. By means of the colorimetric test the relative amounts of iron extracted from soils and clays after ignition for various times was measured. In nearly all the soils and clays examined the solubility of iron in acid was on the decline before 10 minutes of ignition over a bunsen flame. Only in one experiment with a very fat clay did the increase continue for as long as 10 minutes. The following arbitrary figures are typical.

Table II.

Relative amounts of Fe extracted by N/HCl from soils ignited for different times.

	Unignited	5 mins ignition	10 mins ignition
A	1.0	2.8	1.9
B	1.0	3.2	2.5
C	1.0	2.5	1.0

(e) One of two equal portions of a clay subsoil was partially ignited. Each portion was then extracted with $N/5$ HCl. Equal volumes of the filtrates were boiled, cooled and titrated with $N/5$ NaOH using first methyl orange and then phenolphthalein. The difference between the methyl orange reading and the phenolphthalein reading was then taken as a measure of the acid-salt-forming bases (Fe_2O_3 and Al_2O_3) in solution, and the difference between the phenolphthalein reading and the titre of the original acid was taken as a measure of the neutral-salt-forming bases which had gone into solution.

The experiment was also carried out with the same subsoil at its maximum base absorbing power (that is, after extraction with acid as previously described).

The following table shows, in respect of two subsoils, the actual differences of burette readings for 100 c.c. filtrate and the ratios calculated therefrom.

Table III.

Relative amounts of neutral-salt-forming bases (n) and acid-salt-forming bases (a) extracted from two subsoils, before and after ignition, by N/5 HCl.

		Before ignition			After ignition		
		Burette readings		Ratio $n : a$	Burette readings		Ratio $n : a$
		n	a		n	a	
A	Original subsoil	19.2	3.6	1 : 0.19	10.8	15.6	1 : 1.43
	Extracted „	3.2	7.2	1 : 2.25	5.6	32.0	1 : 5.70
B	Original subsoil	30.8	9.2	1 : 0.30	21.2	40.4	1 : 1.91
	Extracted „	9.2	16.0	1 : 1.74	11.2	47.2	1 : 4.21

Clearly therefore the solubility of the acid-salt-forming bases is increased by ignition to a much greater extent than the neutral-salt-forming bases. Indeed the neutral-salt-forming bases dissolve to a less extent after ignition of the original subsoils.

2. The thiocyanate test was applied to a number of soils before and after a partial ignition. Soils containing excessive amounts of organic matter or of chalk showed no reaction with alcoholic thiocyanate after ignition, but with 30 mineral soils taken at random the reaction with thiocyanate was increased. Those soils which gave no colour before ignition did so after, and those which gave a colour before ignition gave a much more intense colour after.

3. The effect of partial ignition on the base absorbing power, as measured by the Hutchinson-McLennan "lime requirement" method, was examined in a number of experiments. Two 20 gm. portions of each sample were weighed out and one portion ignited over a bunsen flame for 5 minutes. "Lime requirement" determinations were then made on the original and on the partially ignited portions. The results are shown in Table IV.

Table IV.

The effect of 5 minutes' ignition on the "lime requirement" of soils and subsoils.

(CaCO_3 per 100 of Soil.)

	Medium soil (sour)	Medium soil (not sour)	Peaty soil	Medium subsoil	Same subsoil after extraction with $\text{N}/2 \text{ HCl}$	Heavy subsoil	Same subsoil after extraction with $\text{N}/2 \text{ HCl}$	Sour grassland soil	Silty coal mea- sures subsoil
Before ignition	0.28	Nil	0.41	0.04	0.78	Nil	1.36	0.11	Nil
After ignition	0.13	Nil*	Nil*	0.07	0.42	0.16	0.60	0.01	0.06

* The concentration of the bicarbonate solution was increased.

Normal soils showed a lower base absorbing power after partial ignition and this effect was very much greater when much organic matter was present. Subsoils containing no organic matter showed a greater base absorbing power after partial ignition. The same subsoils, however, after extraction with dilute acid showed a lower base absorbing power.

4. A number of experiments were carried out with pure substances in search for some synthetic system of known constitution which also

showed an increment in the amount of iron dissolved in dilute acid after partial ignition.

(a) Ferric oxide or hydroxide was obtained in a variety of ways. Two purchased samples (one red and one brown), the dried precipitates obtained by adding NH_4OH , NaOH , and KOH to solutions of FeCl_3 both in hot solution and in the cold, and the gels obtained by evaporating ferric hydroxide sols were all used. They showed a continuous decrease in the solubility of iron in $N/5$ HCl during ignition.

(b) Three 50 gm. portions of ferric oxide were weighed out. One was shaken with 100 c.c. distilled water and then treated with 200 c.c. $\text{Ca}(\text{OH})_2$ solution. Another was shaken with 100 c.c. silica sol (containing 2.085 gm. SiO_2 in the 100 c.c.) and precipitated with 200 c.c. $\text{Ca}(\text{OH})_2$ solution. The remaining portion was shaken with 100 c.c. silica sol and 200 c.c. distilled water were added. Each of these systems was then evaporated to dryness on the water bath, and the relative amounts of iron extracted by $N/5$ HCl before and after 2 minutes' ignition was observed. The ignition decreased the amount of iron dissolved where only $\text{Ca}(\text{OH})_2$ had been added, but increased it about threefold in the two other cases.

The amount of iron dissolved from the unignited material in which silica had been used was very much less than where only $\text{Ca}(\text{OH})_2$ was used.

When the silica sol or the precipitate formed by adding $\text{Ca}(\text{OH})_2$ thereto, was evaporated separately from the ferric oxide and then intimately mixed with it, there was no increase by ignition, but always a decrease, in the amount of iron dissolved by dilute acid.

These experiments were repeated many times with three different samples of ferric oxide, and with similar results.

An increase in the amount of iron dissolved by dilute acid was also observed in the products obtained by suspending ferric oxide in a dilute solution of waterglass and adding a solution of AlCl_3 , and by suspending ferric oxide in AlCl_3 and adding NH_4OH .

Wherever the ferric oxide particles were cemented together by gelatinous precipitates, a partial ignition increased the amounts of iron dissolved by acid, just as is observed in soils.

DISCUSSION.

The flocculation of particles other than soil particles.

The only substances, in the random collection examined, which showed the same anomalous flocculation by calcium hydroxide, as clay, were certain phosphates. Suspensions of the phosphates of aluminium and of iron, as well as some natural calcium phosphates, are flocculated by calcium salts very much better from alkaline than from neutral suspensions.

The similar behaviour of clay and of these phosphates seems to lend support to the explanation of the abnormal flocculation by calcium hydroxide which has been advanced in the earlier papers. Calcium salts added to a dilute ammoniacal solution of a phosphate, produce a voluminous gelatinous precipitate very similar to that produced in dilute ammoniacal solutions of silica. Further, the phosphates of aluminium, iron and calcium are well known to exist quite commonly in the colloidal gel form. The action of calcium hydroxide on the colloidal surfaces of these phosphates is analogous to its action on the colloidal surfaces of silicates and is susceptible to a similar explanation.

Powdered apatite is crystalline, without colloidal properties, and it does not show the flocculation anomaly either before or after extraction with cold dilute acid.

The colloidal condition of phosphates and silicates is undoubtedly related to their solubility and to their availability to the plant. This aspect of the subject is discussed in a separate paper.

The respective rôles of the core and of the colloidal surface.

Assuming the truth of the earlier deduction that calcium hydroxide has a dual action on soil particles (a deflocculating action on the cores of the particles and a precipitating action on the emulsoid surface) it is clear that the deflocculating action may dominate the precipitating action in at least two circumstances:

(i) When the amount of emulsoid matter is small relatively to the core.

(ii) When the amount of calcium hydroxide is insufficient to cause a maximum precipitation with the colloidal matter.

The first of these circumstances has already been demonstrated and discussed. The second has now been demonstrated experimentally, for it has been shown that when the concentration of the flocculant is very low clay suspensions are flocculated less readily by calcium hydroxide than

by a neutral calcium salt. In these low concentrations the calcium hydroxide is insufficient to produce a dominating precipitating action on the surface colloids and the deflocculating action on the cores prevails.

The concentration above which calcium hydroxide becomes the better flocculant is much less with a lean clay than with a fat clay. In a lean clay, where the proportion of effective emulsoid matter is low, less calcium hydroxide is required to cause the maximum precipitation. But because the amount of emulsoid matter is relatively small, the superior calcium hydroxide flocculation is never very marked. In a fat clay the relative effective amounts of emulsoid matter is high and more calcium hydroxide is required to cause precipitation. Experiment shows that emulsoid colloids such as silica protect the particles of some substances and stabilize their suspensions. A fat clay contains a large amount of the protective and stabilizing colloid and more colloid is accordingly required to coagulate it, and precipitate the suspension. Also, because there is so much emulsoid matter in the fat clay the abnormal effect of calcium hydroxide, when it is attained, is very pronounced.

This subject may now be considered from an entirely different viewpoint.

If a clay is treated with an excess of calcium hydroxide, it is flocculated. Two or three washings, however, will reverse the flocculation (see Appendix, paragraph 2), but no ordinary amount of washing with pure water will deplete the soil of lime from the "lime requirement" point of view. Again, therefore, it is clear that a certain easily removed excess of calcium hydroxide is necessary to maintain the flocculated condition of wet clay.

Quite crudely, but in order to arrive at a more precise conception, it may be assumed that when lime has been absorbed by a sour clay, there is at one extreme a part of the lime, correcting the sourness, which is not removable by ordinary water washing, and at the other extreme a part, causing flocculation, which is easily removed by water washing.

It might be supposed that the lime which is held irreversibly (or relatively so) has reacted with the core of the particle, while the easily reversed absorption is an action of the colloidal surface. That view, however, is untenable, for experimental evidence has been described to show that by extraction with dilute acid a soil may easily be brought to a maximum base absorbing power. Acid of concentration considerably below normal, will usually remove all the base which can be afterwards replaced by shaking with calcium bicarbonate. Extraction of the soil with more concentrated acid, which will more effectually attack the

minerals of the cores of the particles causes no increase in the base absorbing power of the remaining soil. The absorbed bases are easily soluble in dilute acid and their extraction brings the soil at once, and decidedly, to its maximum base absorbing power. From this it seems justifiable to conclude that the absorption of lime which is measurable by titration methods is solely confined to the surface colloids. The insignificant amount of lime absorbed by powdered granite, after extraction with acid, also goes to show that the unweathered and crystalline minerals have little to do with the absorption of lime under such conditions as those of the Hutchinson-McLennan "lime requirement" determination.

It is not suggested that these minerals are not reactive but that any part they play in base absorption is small. Powdered and extracted crystalline minerals and rocks do show a slight absorptive power. But even this may be due to the colloidal surface, for the grinding of rocks—particularly the wet grinding of rocks—has a decomposing effect similar to weathering. This is apparent from the classical work of Daubrée¹ and the more recent work of Cushman².

The whole of the absorption of lime—reversible and irreversible—is therefore a phenomenon associated with the colloidal surface. Until sufficient lime has been applied to react with that surface, the lime may exercise a deflocculating action because of the combined effects of the decreasing hydrogen ion concentration and the uncoagulated colloid stabilizing the particles.

After a soil has been treated with excess of calcium hydroxide, washing with water readily removes lime at first, but as the amount of lime gets less it becomes more difficult to remove it. The actual process of washing a soil at the pump after treatment with calcium hydroxide until the filtrate no longer colours phenolphthalein, is a long one. The colour produced by phenolphthalein becomes very gradually fainter in successive washings and in such a way as to make it clear that the operation is not one of merely washing away an excess of the hydroxide in solution, like the washing away of hydrochloric acid after treatment of a soil therewith. During the washing, calcium hydroxide is being removed less and less effectively from the colloidal surface in which it is in some way held.

The retention of lime by this colloidal surface seems to fall on the borderline between the physical phenomenon of a reversible absorption and the chemical formation of compounds such as Way's double silicates,

¹ *Compt. Rend.*, 1857, **44**.

² *U.S. Dept. Agric. Bur. Chem. Bulletin*, 1905, **92**.

which are slowly hydrolysed on washing. The full discussion of the retention of lime by soils is therefore impossible until more is known about these borderline phenomena on the one hand and about the actual constitution of the soil colloids on the other. Meanwhile it seems clear that

- (i) The absorption of lime is mainly confined to the colloidal surface.
- (ii) The absorptive power must be largely satisfied before lime causes the abnormal flocculation of clay.

The partial ignition of soils.

Ignition and solubility. Quite independent evidence that the particles making up the soil aggregates are bound by the cementing action of their gelatinous surfaces, is found in the experiments on the ignition of soils. The experiments show in the first place that partial ignition normally increases the amounts of iron, aluminium, etc. which are extracted by dilute acid. This increase can only be due to one of two general causes. Either the elements concerned are transferred by ignition to some other chemical combination or to some other physical state of higher solubility in acid, or else the amount of effectively exposed surface is increased. The formation of more soluble compounds is most improbable. It is scarcely likely that ferrous iron, ferric iron, aluminium and phosphorus would all be converted to more soluble compounds, and in any case the formation of more soluble forms of iron and aluminium compounds by ignition is the very opposite of usual experience.

Ferric hydroxide powder obtained in a variety of ways failed to show any increased solubility on ignition. When, however, the particles of ferric hydroxide were suspended in a suitable solution and precipitated so that they became aggregated by some gelatinous substance such as silica or alumina, the first effect of ignition was to increase the amount of iron dissolving in acid.

The conclusion is that the increased dissolution is due to the drying up of the colloidal matter, the separation of the particles and the consequent exposure of a larger surface to the acid subsequently added.

This increase in the amount dissolved is not a true increase in solubility. Ignition depresses the solubility of iron, etc. In the early stages of ignition, however, the effect of increased surface more than counteracts that depression of solubility.

Ignition and base absorbing power. The base absorbing power of various soils and subsoils, before and after 5 minutes' ignition, was examined by the Hutchinson-McLennan method. The results recorded in Table IV present three cases for consideration.

1. *Subsoils containing no organic matter and from which the absorbed bases have been removed* by dilute acid, show a *decreased* base absorbing power after partial ignition. This is the simplest and most obvious case. It has been previously argued that the power to absorb base rests chiefly with the colloidal gel matter of the soil. This colloidal matter will be dehydrated and its colloidal state (eventually) destroyed by ignition. In accordance with the argument it is found that an early effect of ignition is the depression of the base absorbing power.

2. *Untreated subsoils, containing no organic matter, but containing absorbed lime*, show an *increased* base absorbing power after 5 minutes' ignition. The inference is as follows. In the original unignited material the whole of the absorptive power of the surface colloids of the subsoil *aggregate* is satisfied or nearly so. On ignition the aggregate is disrupted by the shrinkage of the colloids and in the first stages of such disruption new surfaces, whose absorptive power is not satisfied, are exposed. Such new surface will lose its absorbing power as ignition proceeds, but in the first stages of ignition the development of new surface more than counteracts the destruction of its colloidal state. The subsoil aggregate may show no lime requirement at all *as an aggregate* for the absorptive power of the outer surface of the aggregate may be fully satisfied with lime. When the aggregate becomes broken the surfaces of *the particles* are exposed and their exposure takes place before their absorptive power is lost. After a short ignition, therefore, a subsoil free from organic matter and which originally has no base absorbing power shows a base absorbing power.

This consideration fully supports the earlier views that lime reacts with the colloidal surface forming a precipitate which entangles and binds the particles. The complete formation of such an absorption compound will clearly take place only over the outer surface of the resulting aggregate.

This view is also in accordance with the fact that after ignition it is the acid-salt-forming bases rather than the neutral-salt-forming bases which dissolve to a greater extent in acid.

3. *Soils containing organic matter* show a marked *decrease* in base absorbing power after partial ignition. Now, humus undoubtedly plays an important part in the absorption of lime by the soil colloids. On ignition its colloidal state will be destroyed very rapidly. Where humus is present therefore the decline in base absorbing power on ignition will at first be very rapid and may easily overbalance the increase due to new surface exposure. Moreover, the lime absorbed by the humus will

presumably be liberated during the combustion of the humus and remain in the residue as free base.

These considerations seem to afford an adequate explanation of the experimental facts that the base absorbing power of soils containing organic matter falls during partial ignition and that the fall is very great when much organic matter is present. The original peaty soil had a high "lime requirement," but after 5 minutes' ignition it increased the concentration of the bicarbonate solution very considerably.

It will be noted that normal mineral soils, after partial ignition, show a lower base absorbing power but an enhanced reaction with thiocyanate. The reason of this is quite obvious in view of the fact that the proportion of the iron and aluminium to the calcium which present themselves for dissolution in acid is increased by partial ignition. The actual power to absorb lime from bicarbonate, and presumably potassium from potassium thiocyanate, is less, but after the potassium has been absorbed the free thiocyanic acid attacks a relatively greater proportion of iron and aluminium.

SUMMARY.

In support and extension of the conclusions drawn in the earlier papers the following facts and deductions are submitted:

1. The only sparingly soluble substance, from a random collection examined, whose suspensions showed the same abnormal flocculation by calcium hydroxide that is shown by clay, were certain phosphates of iron aluminium and calcium. The abnormal flocculation of these phosphates is open to an explanation quite analogous to that already advanced for the flocculation of clay.

2. Until the amount of calcium hydroxide added to a suspension of clay or phosphate reaches a certain amount its abnormal flocculating power is not manifested. The amount required to produce the abnormal flocculation is greater for a fat clay than for a lean one. This is in agreement with the view that the abnormal flocculation is caused by a coagulation of emulsoid matter, for obviously such coagulation will not become dominant until a sufficient amount of the precipitant has been added.

3. The lime absorbed by a soil can be wholly and completely removed by a dilute acid treatment which cannot very appreciably decompose the unweathered minerals. It is therefore concluded that the absorption of lime by a soil is an absorption by the soil colloids and not by the unweathered minerals.

4. The ignition of a soil for a few minutes over a bunsen flame increases the amounts of iron and aluminium dissolved by acid. Evidence is brought to show that this is due to a destruction of the colloids which bind the particles together, and a consequent exposure of a larger surface.

5. The effect of a partial ignition on the base absorbing power of soils and subsoils is described and the results are claimed to be in agreement with the view that the particles in the aggregates are bound together by gelatinous colloidal matter.

APPENDIX.

The preparation of clay suspensions.

For experimental work on flocculation it is frequently necessary to prepare clay suspensions without the use of excess of ammonia solution which is employed in routine mechanical analysis. Two notes on the making of such suspensions are appended:

1. In order to obtain, after sedimentation, the maximum amount of clay in suspension it is necessary to adjust the relative amounts of soil, etc. and water. All soil and clay particles are largely aggregated. If therefore the proportion of clay to water is very large the deflocculated particles are entrained by the aggregates and the whole settles down leaving clear water above. On the other hand, if the proportion of clay to water is small the amount of clay in suspension will be small.

This can be strikingly demonstrated by arranging a series of tubes containing a large amount of a stiff clay at one end and gradually decreasing amounts through the series. If water is then added to the same height in each tube, it is found after agitation that the supernatant liquid is quite clear in a few minutes where the amount of solid is greatest. It becomes gradually more turbid down to a certain point in the series beyond which it becomes less turbid again.

Only by such trial can the most suitable relative amounts of solid to water be ascertained.

2. An almost neutral suspension of clay is easily prepared by extracting the soil with dilute acid, washing, agitating with excess of lime water, filtering at the pump and thoroughly washing with hot water. The *wet* soil will then give good clay suspensions.

Soils treated in this way, or after extraction with acid, should not be dried after washing if a maximum clay suspension is required.

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ON THE RELATIVE GROWTH AND DEVELOPMENT OF VARIOUS BREEDS AND CROSSES OF PIGS.

BY JOHN HAMMOND, M.A.

(Institute of Animal Nutrition, School of Agriculture, Cambridge.)

INTRODUCTION.

Few reliable figures (1) exist at the present time which show the relative qualities of the various British breeds of pigs in respect of their ability to put on weight and the relative proportions of meat and offal in the carcasses. Weights and carcass percentages of German breeds have been published by the German Agricultural Society (2).

An investigation based on the records of the Smithfield Club's Fat Stock Show was therefore undertaken to determine the relative merits of the various breeds.

Hitherto most of the work which has been done on the growth and fattening of farm animals has been based on the chemical composition of the body; this, however, does not altogether show the economic side of the question as it fails to differentiate between parts of different value and edibility.

In all interpretations of the results of this investigation given below it should be remembered that the animals exhibited were all in a fat condition and that the Show is essentially a butchers' show. The averages quoted for the different breeds will be maximum averages for the best animals of the breed; the commercial specimens of the breed would normally fall below this average but there is no reason to suppose that the relative positions of the breeds would be affected thereby.

The results in general show the rate of growth and carcass percentage of the different breeds at 3, 5, 7, 9 and 11 months old; data on the rate of maturity is also presented as well as figures which show the changes that have been made in breeds during the last few years.

In order to determine the actual amount of edible meat produced it is necessary also to know the weights of muscle, fat and bone in the carcass, but there are no data on this point in the records of the Show and this remains to be discussed in another investigation.

388 *Growth and Development of Breeds and Crosses of Pigs*

The available published data on the proportions of meat and bone in the carcase, collected from the literature, is given below from which it would appear that the proportion of bone varies in different breeds and decreases with age. Cornevin(3) has shown that the live weight of the wild boar contains 3.9 per cent. of bone whereas the Craonnaise breed contains only 2.6 per cent. Long(4) quotes experiments by McMurtree who found that the carcase of a Poland China 11 months old and weighing 340 lbs. contained 8.4 per cent. bone, 39.4 per cent. muscle and 45.5 per cent. fat, whereas a Berkshire 9 months old and weighing 245 lbs. contained 9.3 per cent. bone, 42.7 per cent. muscle and 41.6 per cent. fat. Wellman(5) found that a Berkshire 3 weeks old and weighing 10 lbs. contained 13.5 per cent. bone, whereas one of 9 weeks old and weighing 26 lbs. contained 11.9 per cent. bone and a Large White of about 15 months old and weighing 349 lbs. contained 9.1 per cent. bone. The following figures have been calculated from data published by Tschirwinsky(6).

No.	Breed	Age weeks	Live weight lbs.	% of skeleton
1.	Windsor	10	16.1	18.6
2.	"	28	75.1	10.3
4.	Large White	9	24.3	17.0
3.	"	23	54.6	13.5

While some of the discrepancies between different authorities may be accounted for by the various methods adopted in cleaning the skeleton before weighing, yet on the whole the proportion of meat to bone varies considerably both with breed and with age.

Material and Methods.

The investigation was conducted in the same way as that previously described for cattle(7) and sheep(8) and consisted of a statistical treatment of the records of the Fat Stock Show held by the Smithfield Club at Islington from the year 1901 (when the classes for pigs were restarted) until the year 1913 inclusive. I am indebted to Mr E. J. Powell the Secretary of the Club who has kindly supplied me with these records.

Two series of competitions for pigs exist (1) The Live Classes, and (2) The Carcase Classes.

In the first Series—*Live Classes*—a record is kept only of the age and gross weight of the animals. This series is divided into classes for breeds and crosses of different ages, the sex not being specified, each pen consisting of two pigs. Classes also exist for porkers not exceeding 100 lbs. weight in which the age is not limited. In addition there are classes for single pigs of the different breeds under 12 months old.

In the second series—*Carcase Classes*—which were started in 1903, a record is kept not only of the live weight and age of the pig but also the weights of the carcase and pluck after the animal had been killed. This series is divided only into classes for pigs of different weights; in some cases in addition there being an age limit as well. Each entry consists of a single pig only. The entries for this series are very small compared with the number of exhibits in the live classes especially in view of the fact that the carcase test is the ultimate object for which the pigs were bred.

The weights of the animals and carcasses have been carefully taken by the officials of the Club under the supervision of the Stewards. For the details of the methods of slaughter and dressing of the carcasses I am indebted to Mr Charles Bone who has been responsible for these and who has kindly furnished me with information on these points.

Neither in the Live Classes nor in the Carcase Classes is there any specification of sex. Henry (9) found that boar pigs weighed slightly more than sows, but Danish (10) experiments have shown very little difference between the sexes.

The details given in the records of the Show have been treated statistically so as to give information on the points it was desired to investigate. Weights throughout have been given in lbs. and decimals of a pound, and ages in months and weeks.

As the single pig classes would naturally attract the exceptional animals it was thought better to keep them separate. The fact that in the porker classes the weight was limited but not the age was not considered to be of sufficient importance to warrant separate treatment and they have been grouped by their age; breeds maturing early would be exhibited in this class at a younger age than those of the late maturing breeds.

In order to avoid confusion when discussing the results of the investigation below, the following account gives the methods by which the various tables in the text have been compiled.

Table I has been prepared from the records of the Show direct. As each pen consists of two pigs the total has been halved to obtain the weight of individual pigs. The divisions into age groups correspond more or less with classes at the Show, the bulk of the porkers falling into the "under 3 months" and "3-5 months" groups while the majority of the classes "not exceeding 9 months" and "not exceeding 12 months" fall into the groups "8-9 months" and "10-12 months" respectively. A few of the former constitute part of the "6-7 months" group. The single pig

390 *Growth and Development of Breeds and Crosses of Pigs*

classes have been kept separate and are shown at the end of the table. Averages calculated from less than 10 individuals are shown in italic type.

Table II has been calculated from *Table I* by dividing the weight by the number of weeks old (counting 4 weeks to a month). The weight at birth, owing to lack of data on this point, has been taken as zero. The numbers of individuals from which the results have been calculated are also given so that an estimate can be made of the reliability of the average. Averages calculated from less than 10 individuals are shown in italic type.

Table III has been compiled from *Table I* by adding or subtracting the number of weeks growth (see *Table II*) required to correct for age. Averages calculated from less than 10 individuals are shown in italic type. The probable error of the mean has been calculated in several cases and is shown in *Table IV*.

Table A. Probable error of mean (pen of two), lbs.

Months old—	3	5	7	9	11
Large White	3.63	3.48		4.36	5.12
Berkshire	2.76	1.36	12.89	0.98	2.51

Table IV has been prepared from the records of the Carcase Classes direct. Results averaged from less than 5 individuals are shown in italic type. The parts into which the pig is divided on slaughter are as follows:

Carcase. Includes the head, feet and skin (but not hair or claws). The skin varies in thickness in different types of pigs(11). Henseler(12) in a starved Bavarian belted sow of 79 lbs. found that it was 14 per cent. of the live weight and Colin(13) that it was 10.1 per cent. in a pig weighing 64 lbs. Within a breed the proportion would probably decrease with the increasing size of the animal.

Pluck. Consists of the pluck proper—heart, liver, lungs, diaphragm, oesophagus and trachea—and in addition the canal fat, thus differing from the pluck of cattle and sheep. The total percentage is very similar to that found by Holm(14) in Danish pigs of the same weight.

“Unaccounted for.” This is the difference between the sum of the two foregoing parts and the live weight; it consists mainly of stomach and intestines and their contents, but also includes blood, hair, spleen, pancreas and genitals as well as the weight lost on cooling.

In order to give some idea of how the weight of the Pluck and “Unaccounted for” is distributed among the different organs the following *Table B* has been prepared:

Table B. *Organs as percentage of live weight.*

Authority	Breed	Age	Live weight lbs.	Pluck						Unaccounted for										
				Heart	Lungs	Liver	Diaphragm	Trachea	Esophagus	Total	Cooling	Hair and claws	Stomach (a)	Intestines (b)	Contents of (a) and (b)	Blood	Genitals	Pancreas	Spleen	Total
Lowrey ⁽¹⁵⁾	?	Fœtus	1½	1.0	2.0	3.1	—	—	—	—	6.1	—	—	3.6	2.4	2.4	—	—	—	6.0
Wellman ⁽⁶⁾	Berkshire	3 weeks	10	2.3	—	3.7	—	—	—	—	6.0	—	2.1	7.0	2.8	5.9	0.2	—	0.3	18.3
Wellman ⁽⁵⁾	Berkshire	9 weeks	26	2.2	—	3.2	—	—	—	—	5.4	—	1.2	5.7	5.4	5.4	0.3	—	0.2	18.2
Henseler ⁽¹²⁾	Bavarian belted	9 months	Starved 58	0.5	1.1	2.6	0.1	—	0.2	—	4.5	—	—	1.2	4.9	11.5	3.4	0.3	0.2	21.6
Colman ⁽³⁾	?	?	64	0.6	0.9	3.1	—	0.9	—	—	5.5	—	0.1	1.2	4.2	2.5	2.7	1.0	0.2	12.3
<i>The Fidd</i> ⁽¹⁶⁾	?	?	128	3.1	—	1.6	with heart	—	—	—	4.7	—	3.1	8.6	—	—	3.9	—	—	15.6
Plimmer ⁽¹⁷⁾	?	?	145	—	—	2.9	—	—	—	—	2.9	3.1	—	7.7	—	—	2.1	—	—	12.9
Lowrey ⁽¹⁵⁾	?	?	225	0.3	1.4	0.7	—	—	—	—	2.4	—	—	4.8	2.0	—	—	—	—	6.8
Henseler ⁽¹²⁾	Bavarian belted	9 months	279	0.3	0.5	1.9	0.05	—	0.1	—	2.8	—	—	0.4	2.9	2.9	2.9	0.6	0.1	9.9
Wellman ⁽⁵⁾	Large White	About 15 months	319	1.8	—	1.0	—	—	—	—	2.8	—	—	5.7	2.8	2.2	—	—	0.1	10.8

The differences between authorities may be due partly to variations in method but are largely affected by differences in the size and age of the animals. The liver of the pig at birth is much larger in proportion (3 per cent. of liver) than it is at a year old (1 per cent. of liver). It will be noticed too that the proportion of the stomach and intestine contents increases from about 2.5 per cent. at birth to about 11 per cent. at 3 months old and then decreases again to about 2.5 per cent. at a year old.

Table I has been calculated from *Table IV* in the same way that *Table II* has been prepared from *Table I*. Averages from less than 5 individuals are shown in italic type. The four breeds from which the average at the base of the table has been calculated are as follows: Large White, Large Black, Berkshire and Middle White. The breed average only has been considered and not the number of individuals in each breed.

Table VI has been prepared from *Table IV* in the same way as *Table III* has been calculated from *Table I*. Averages from less than 5 individuals are shown in italic type. The average at the base of the table refers to the same four breeds as in the preceding table.

Table VII has been compiled from *Table VI* by calculating the weight of the carcass or part as a percentage of the live weight. Averages from less than 5 individuals are shown in italic type. The average at the base refers to the same four breeds as stated in *Table V*.

Table VIII has been calculated from the averages of the four breeds given at the base of *Table VI*. The method used is described in the text (see p. 402).

Table IX has been prepared from *Table III* by calculating the weights at various ages as percentages of the weights at 11 months old. The single pig classes at 9 months are shown as a percentage of the single pig classes at 11 months. Figures calculated from less than 10 individuals on either side are shown in italic type.

Table X has been compiled from *Table VI* by calculating the weights at various ages as percentages of the weight at 11 months old. Figures calculated from less than 5 individuals on either side are shown in italic type.

Table XI has been prepared from the Live Class records of the Show direct. All doubtful and second cross animals have been eliminated. Averages calculated from less than 10 individuals are shown in italic type.

Table XII has been calculated from *Table XI* in the same way that *Table II* has been calculated from *Table I*. Figures from less than 10 individuals are shown in italic type.

Table XIII has been compiled from *Table XI* in the same way that *Table III* has been prepared from *Table I*. The mean weights between the two breeds have been taken from *Table III*. Averages from less than 10 individuals are shown in italic type.

Table XIV has been calculated from *Table XIII* in the same way that *Table IX* has been prepared from *Table III*. Figures calculated from less than 10 individuals on either side are shown in italic type.

Table XV has been prepared from the records of the Carcase Classes direct. All second and doubtful crosses have been eliminated. All figures are shown in italic type as in no case is the average calculated from more than 5 individuals.

Table XVI has been compiled from *Table XV* by showing the percentage of the carcase or part as a percentage of the live weight. All figures are given in italic type as in no case is the average calculated from more than 5 individuals.

Table XVII has been prepared from the records of the Live Classes of the Show after correction of each for age as described for *Table III*. Figures calculated from less than 10 individuals are shown in italic type.

Table XVIII has been compiled from the records of the Carcase Classes after correction of each for age as described for *Table III*. Averages from less than 5 individuals are shown in italic type.

Table XIX has been calculated from the records of the Live Classes of the Show after the weight of each pen of 2 pigs had been corrected for age as described in *Table III*.

Table XX has been calculated from the records of the Carcase Classes of the Show after the weight of each animal had been corrected for age as described in *Table III*.

Table XXI has been compiled from the records of the Carcase Classes of the Show by calculating for each animal the carcase and parts as a percentage of the live weight. Individuals were then classified into three equal groups—high, average and low—according to the live weight or percentage of the part they were grouped by. The averages for each of these groups were then calculated. Each different age group as shown in *Table IV* was treated separately in this way and then all the age groups were combined and their average is given in this table. The numbers of animals dealt with in each group were as follows: high 74, average 75, low 75.

RESULTS.

The results obtained are considered in sections below under various headings which it was considered might have an effect on growth and development—breed, age, early maturity, cross-breeding, selection, individual variation and correlation. In general, the results obtained from the Live Classes for actual gross weight have been considered first followed by the proportional development and weights of the various organs as obtained from the Carcase Classes.

Breed. No satisfactory explanation has yet been offered why one animal or breed should produce meat more economically than another. Armsby and Fries(18) have shown that it does not depend on differences in digestive power; their experiments indicated that the well-bred animal had a lower maintenance requirement than a scrub, due to the latter's more nervous disposition and greater restlessness.

Feeding tests for the economy of gain in different breeds of pigs made at the Ontario College of Agriculture(19) have failed to show any uniform difference between breeds as so much depended on the strain used. Carlyle(20), however, who compared Berkshires with Razorbacks or semi-wild swine found that the latter required more feed per unit gained. Similar results were obtained by Diffloth(21) when comparing Suffolk pigs with those of a German breed.

It is rather in the utilization than in the absorption of food that breeds differ; variations in utilization will be seen below. Little or nothing is known as to whether the underlying physiological differences in the metabolism of different breeds are due to variation in heat loss by the skin as shown by Wood and Hill(22) or to differences in the glycogenic coefficient of the blood controlled by the glands of internal secretion. Lühring(23) states that it is possible to distinguish between the different breeds of pigs by the protein differentiation method.

Table I shows the average ages and weights of the different breeds of pigs as compiled from the records of the Live Classes. The following Table II shows these weights translated into lbs. per week gained since birth. The weight at birth has been neglected owing to the absence of data for the different British breeds. Carmichael and Rice(24) found that the average birth weights of breeds in America ranged from 2.25 lbs. for Duroc-Jerseys to 2.61 lbs. for Berkshires but it was considerably affected by other factors such as litter size, sex, age of sow, etc. Meek(25) states that the birth weight varies from 1.5 lbs. to 4.5 lbs. and Henry(26) that the variation is from 1.3 lbs. to 3.4 lbs.

Table III. *Comparative weights of different breeds of pigs—lbs.*

Age in months	Pen of 2 classes				Single pig classes		
	3	5	7	9	11	9	11
Lincolnshire Curly Coated	71		311	404	510	165	183
Large White	86	107		390	481	421	490
Large Black			305	371	470		470
Somersetshire		138		316	430		435
Tamworth			297	334	414	349	404
Berkshire...	85	116	218	337	402	327	404
Middle White	77	118	210	325	384		385
Small Black					379		376
Dorset						238	349
Small White			151	216	273		

In Table I it will be seen that there is variation in the average age at which the different breeds were exhibited so that the figures are not strictly comparable.

Corrections have therefore been made for this from the rate of growth shown in Table II and the comparative weights of breeds at the common ages of 3, 5, 7, 9 and 11 months are given in Table III and it is to this table that reference is made below.

The breeds in Table III are arranged in order of their weight at 11 months old, the mature size of the breed; the differences in the relative order at the other ages are due to variations in the rate of maturity and will be discussed below.

The Lincolnshire Curly Coated (averaging 510 lbs.) is the heaviest breed with the Large White next some 30 lbs. lighter while the Large Black follows closely weighing some 10 lbs. less.

There is then a rather large drop of 50 lbs. to the Tamworth, with the Berkshire some 10 lbs. below and the Middle White about 20 lbs. lighter still. There is then a very big gap of some 110 lbs. to the now almost extinct Small White.

A few animals described as the Somersetshire breed (probably Gloucester Old Spots) come midway between the Large Black and the Tamworth in size; while a few called Small Blacks and Dorsets are slightly lighter than the Middle Whites.

Much work on the comparative slaughter weight of pigs has been done by Hofman-Bang, Mørkeberg and Lund(27) in Denmark and has been mainly directed to testing two breeds, the Large White and Native Danish and their crosses. These tests, which give data of gain in live weight per unit of food consumed up to the age of approximately 200 days and 200 lbs. live weight, conclude with carcase weights at that age only and show consistent results in that the carcase weight of the Large White

is about 2 per cent. greater than that of the Native Danish. The investigation which was practical in nature was however limited to pigs of this age, so does not show differences due to age.

Sennler⁽²⁸⁾ found that the carcase weights of Berkshires were on the average higher than those of German native breeds of the same type. Henry⁽²⁹⁾ quotes from Cuvier that the intestine length of the wild boar is 9 times the body length whereas that of the domestic boar is 13.5 times and he found himself that in fat hogs it was 21 times. No weights are given, however, and it does not follow that increased length means increased weight; if the diameter were decreased the absorptive area would still be greater and the weight of contained foodstuffs the same or less. Henseler's⁽³²⁾ data shows that a starved pig of 9 months old had an intestine length 18.1 times the body length, whereas in a well-fed pig of the same age and from the same litter the length was only 16.9 times; moreover, the intestine was 4.95 per cent. of the live weight in the starved animal, whereas it was only 2.8 per cent. in the well-fed.

The average weights of pigs killed in the carcase competitions are shown in Table IV which also gives the numbers exhibited and their average age. These figures have been translated into lbs. per week increase which are shown in Table V. The birth weight has been neglected as the weights of the different breeds at birth were not known. Owing to differences in age the weights of the different breeds given in Table IV are not strictly comparable. Table VI has therefore been prepared to show all breeds calculated to common ages.

If the live weights of animals exhibited in the "Carcase Classes" given in Table VI are compared with those shown in Table III for the "Live Classes" of the same age it will be seen that, as has been pointed out by Long⁽³⁰⁾, the live class animals are much heavier. With Berkshires the difference at 3 months is 12 lbs., while at 11 months it is 154 lbs. and again with Large Whites at 3 months the difference is 19 lbs., while at 11 months it is 156 lbs. The reason for this is probably that the "Live Class" animals are more heavily fattened. Evidence, which it is hoped to publish shortly, has been accumulated on this point in sheep and it shows that the difference in weight and composition of the body of animals from the Live and Carcase Classes is one of fat alone. It would seem probable that this result would also apply to pigs and that the difference in weight between the two classes is due to extra fat in the Live Classes, which from the butchers' point of view is superfluous. It is worthy of note that the Tamworth, a breed which does not put on superfluous fat easily, has only a difference of 45 lbs. between "Carcase"

Table V. *Carcase Classes—rate of growth in lbs. per week.*

Breed	2—3 months				4—5 months				6—7 months				8—9 months				10—11 months			
	Live	Carcase	Pick	Unaccounted For	Live	Carcase	Pick	Unaccounted For	Live	Carcase	Pick	Unaccounted For	Live	Carcase	Pick	Unaccounted For	Live	Carcase	Pick	Unaccounted For
Middle White	4.98	3.69	0.30	0.99	4.70	3.71	0.24	0.85	6.89	5.67	0.31	0.90	7.85	6.39	0.31	0.94	6.41	5.18	0.25	0.70
Berkshire	6.09	4.69	0.33	1.05	5.11	4.51	0.28	0.85	6.25	5.07	0.30	0.88	6.43	5.31	0.29	0.84	5.64	4.69	0.25	0.71
Large White	5.76	4.20	0.34	1.22	6.22	4.79	0.33	1.10	8.23	6.66	0.30	1.18	7.23	5.87	0.33	1.03	7.38	6.16	0.30	0.92
Tamworth	—	—	—	—	5.35	3.75	0.32	1.27	5.87	4.48	0.30	1.09	5.73	4.50	0.27	0.87	8.38	7.11	0.33	0.91
Large Black	6.71	4.89	0.39	1.43	5.51	4.11	0.34	1.06	7.73	6.16	0.38	1.19	6.77	5.44	0.33	1.01	5.91	4.77	0.30	0.81
Lincolnshire (Curly Coated)	7.23	5.85	0.31	1.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Average (4 breeds)	5.89	4.37	0.34	1.17	5.38	4.28	0.30	0.96	7.27	5.89	0.34	1.04	7.07	5.80	0.31	0.95	6.34	5.27	0.27	0.76

Table VI. *Comparative carcase weights of different breeds of pigs, lbs., 1903-13.*

Breed	3 months				5 months				7 months				9 months				11 months			
	Live	Carcase	Pick	Unaccounted For	Live	Carcase	Pick	Unaccounted For	Live	Carcase	Pick	Unaccounted For	Live	Carcase	Pick	Unaccounted For	Live	Carcase	Pick	Unaccounted For
Middle White	59.8	41.4	3.6	11.8	94.1	72.3	4.9	16.9	192.8	158.8	8.7	25.3	282.6	237.4	11.3	33.9	283.1	241.2	11.0	36.9
Berkshire	73.1	56.3	4.0	12.5	102.2	80.5	5.6	16.1	175.1	142.0	8.3	24.8	231.9	191.0	10.4	30.2	248.4	206.3	11.0	31.1
Large White	67.4	49.2	4.0	14.2	124.5	95.8	6.7	22.0	230.4	186.4	10.9	33.1	260.1	211.4	11.7	36.9	324.7	271.2	13.1	40.4
Tamworth	—	—	—	—	107.0	75.0	6.5	25.5	161.4	125.5	8.5	30.4	206.2	165.1	9.8	31.3	368.8	312.9	14.9	41.6
Large Black	80.6	58.7	4.8	17.1	111.2	82.2	6.7	22.3	216.4	172.4	10.6	33.4	243.8	195.7	11.8	36.3	260.1	209.8	13.4	36.9
Lincolnshire (Curly Coated)	86.8	70.2	3.7	12.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Average (4 breeds)	70.2	52.1	4.1	13.9	108.0	82.7	6.0	19.3	203.7	164.9	9.6	29.2	254.6	208.9	11.3	34.3	279.1	232.1	12.1	34.9

400 *Growth and Development of Breeds and Crosses of Pigs*

and "Live" Classes at 11 months old as compared with 151 lbs. and 156 lbs. for Berkshire and Large Whites respectively. At 3 months old the Live Classes are only 16 per cent. heavier than the Carcase Classes, yet at 11 months old this difference is increased to 62 per cent., the young animal being much more difficult to overfatten than the old one.

The carcase percentage for the different breeds is shown in Table VII; the variations at different ages are discussed below (see Age) as also are the relative orders of the breeds at the younger ages (see Early Maturity).

The Middle White has the highest carcase percentage and is followed by the Berkshire, Large White, Tamworth and Large Black in the order named. The proportion of pluck is in inverse proportion to the carcase percentage and so the order of breeds as regards this part is reversed.

The carcase percentages are on the whole less than those of pigs exhibited at the International Live Stock Show at Chicago⁽³¹⁾ which vary from 83 to 88 per cent. but there a different type of pig—the lard pig—is required.

Table VII. *Proportions of organs in different breeds of pigs—*
as percentage of the live weight.

Breed	3 months			5 months			7 months			9 months			11 months		
	Carcase	Pluck	Unaccounted for	Carcase	Pluck	Unaccounted for	Carcase	Pluck	Unaccounted for	Carcase	Pluck	Unaccounted for	Carcase	Pluck	Unaccounted for
Middle White ...	71.3	6.0	19.7	76.8	5.2	18.0	82.4	4.5	13.1	84.0	4.0	12.0	85.2	3.9	10.9
Berkshire ...	77.0	5.5	17.5	78.7	5.5	15.8	81.1	4.7	14.2	82.5	4.5	13.0	83.1	4.4	12.5
Large White ...	73.0	5.9	21.1	76.9	5.4	17.7	80.9	4.7	14.1	81.3	4.5	14.2	83.5	4.0	12.5
Tamworth ...	—	—	—	70.1	6.1	23.8	76.3	5.2	18.5	80.1	4.7	14.9	84.8	4.0	11.2
Large Black ...	72.9	5.8	21.3	73.9	6.0	20.1	79.7	4.8	15.5	80.3	4.8	14.9	80.7	5.1	14.2
Lincolnshire Curly Coated	80.9	4.3	14.8	—	—	—	—	—	—	—	—	—	—	—	—
Average (4 breeds)	74.3	5.8	19.9	76.6	5.5	17.9	81.0	4.7	14.3	82.0	4.4	13.6	83.4	4.3	12.6

Age. The effect of age on the rate of growth in live weight in the different breeds, calculated as increase in lbs. per day since birth, is seen in Table II and V. The rates of growth of two breeds (Berkshire and Middle White) of which sufficient numbers have been available at all ages, have been averaged and are shown in Fig. 1.

The rate of growth in these two breeds falls from 3 to 5 months and then rises till it reaches a maximum at 9 months old when it falls again. The reason for the first drop between 3 and 5 months is to a very small extent caused by neglecting the birth weight which would naturally play

a greater part in pigs of smaller weight; this is not the main reason however which is probably due to the fact that the 3 months old pigs have not been weaned early but forced through, whereas the 5 months ones have often been weaned early and some may possibly have had a short store period.

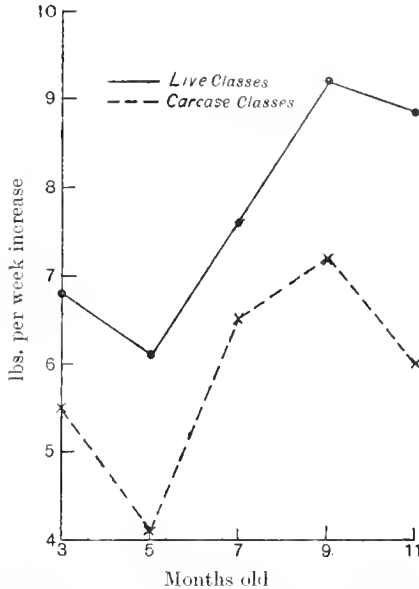


Fig. 1. Rate of Growth—Live Weight—lbs. per week since birth.
Average of 2 breeds (Berkshire and Middle White).

The maximum rate of growth in these two breeds appear to be made at 9 months old but if reference is made to Table II for the Live Classes and Table V for the Carcase Classes it will be seen that breeds vary greatly at which the maximum rate is attained: the Large White and Large Black attaining their maximum at 7 months. It is notable that although the pork type of pig (Middle White, Berkshire) appears to reach the maximum growth later than the bacon type (Large White and Large Black) yet the decline in rate of growth of the latter is not so great between the ages of 9–11 months as it is in the former.

Since live weight growth is the sum of the growth of various organs and tissues in the body it does not necessarily follow that the rate of increase of meat is most at this time. The differences in rate of increase between the Carcase and Live Classes (shown in Fig. 1) which become greater as the pigs get older lead one to believe that the growth at this stage (9 months) consists mainly of fat.

The variations in rate of growth in different breeds have yet to be analysed in terms of the components of the body: the various systems of the body reach their maximum rate of growth at different stages and thus breed variations in the rate of growth may be explained.

The figures given above for rate of growth may be criticized however because they show the rate from birth to that particular age: thus they give the total result of growth to the particular age and not the rate at which growth is proceeding at the time. The rate of growth between certain ages has been calculated in another way—by subtracting the weight at x months from the weight at y months and the age at x months from the age at y months and from this finding the rate of growth per week. This has been done for four breeds and the results are shown in Fig. 2 and in Table VIII. From these it will be seen that, as before, the rate of growth falls between 3 and 5 months but attains its maximum (of approximately 12 lbs. per week) between 5 and 7 months and then falls again until between 9 and 11 months it is only about 3 lbs. per week.

Data collected from various authorities for the growth in live weight of the pig are given in the following table:

<i>Growth in lbs. per week gain.</i>												
Months old	...	1	2	3	4	5	6	7	8	9	10	11 12
Buckley ⁽³²⁾	5.3	8.3	11.1	13.5	—	—	—	—
Harper ⁽³³⁾	2.1	3.5	5.5	7.5	11.0	13.0	10.5	10.0	—	—
Ohio ⁽³⁴⁾	4.9	12.2	12.0	12.7	11.8	—	—	—	—
Ostertag and Zuntz ⁽³⁵⁾	...	2.8	—	—	—	—	—	—	—	—	—	—

The economy of gain in live weight however cannot altogether be measured by the rate of increase per week. Henry and Morrison⁽³⁶⁾ found from data collected from the U.S. Experimental Stations that pigs of 78 lbs. (corresponding to 3 months old) put on 100 lbs. live weight from 400 lbs. food whereas pigs of 320 lbs. (corresponding to 9 months old) put on 100 lbs. live weight from 535 lbs. food: the composition of this gain in live weight as shown in Table VIII however is very different.

Morgan⁽³⁷⁾ has pointed out that man is larger than the rabbit because he grows for a longer period but the daily increases in weight are nearly the same. On the other hand, rabbits attain a larger size than guinea-pigs because they grow faster and not for a longer time. Meek⁽³⁸⁾ too has shown that although a donkey and a Large White pig attain much the same ultimate weight there is a great difference in the form of their growth curve. When one compares the rate of growth of sheep (Murray⁽³⁹⁾) with pigs it will be seen that the maximum rate of daily increase in

weight occurs in the 2nd month in sheep, but not until about the 7th month in pigs. What factor decides the point of maximum daily growth is uncertain; it may be that the maximum comes where the falling curve of the velocity of growth (as measured in lbs. per 100 gained) cuts the ascending curve of increasing body weight¹—Robertson's (40) autocatalytic theory—or that the growth curve is in reality a combined curve

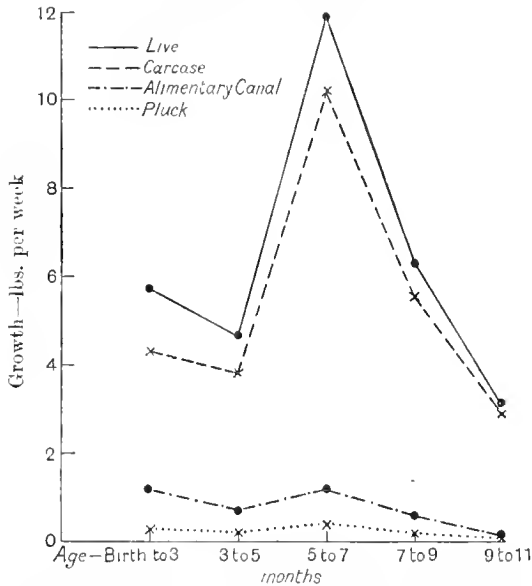


Fig. 2. Rate of Growth—lbs. per week—Carcass Classes.
Weight at birth neglected. (Av. of 4 breeds.)

Table VIII. Rates of growth and carcase percentages of pigs of the Carcase Class.

Age in months	Rate of growth—lbs. per week					Percentage of the live weight				
	Birth to 3	3—5	5—7	7—9	9—11	Birth to 3	3—5	5—7	7—9	9—11
Live weight	5.84	4.74	11.96	6.35	3.07	—	—	—	—	—
Carcase	4.34	3.82	10.27	5.50	2.90	74.3	80.6	85.9	86.6	94.5
Pluck	0.34	0.24	0.45	0.21	0.10	5.8	5.1	3.8	3.3	3.2
Intestines, etc.	1.16	0.68	1.24	0.64	0.07	19.9	14.3	10.3	10.1	2.3

of the growth of different organs, each of which reaches its maximum at a different time; for example, the growth of lambs during the second month of life is above normal owing to the development of the rumen and its contents. Such an explanation would account for the differences

¹ This depending on the relation between the weight of the individual young at birth to the weight of the mother which naturally falls as the number of young per birth increases.

in form of the growth curves of the pig and the donkey and these differences are an exaggeration of what early maturity means in animals. In the guinea-pig Read (11) found that during uterine development cycles of growth took place and the same thing occurs in man when growth is accelerated about the age of puberty (Robertson (12)). These facts indicate that it is impossible, in more than a very general sort of way, to construct growth formulæ to fit the curve of growth of any animal.

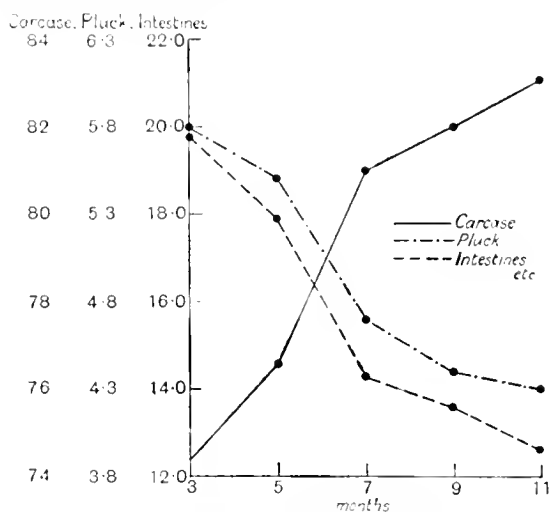


Fig. 3. Proportions of parts—pigs (4 breeds). Per cent. of live weight.

The proportional development of the carcass, pluck and alimentary canal, etc. at the different ages is shown by the average of four breeds at the end of Table VII and is also given in diagrammatic form in Fig. 3. The carcass percentage shows a steady rise from 74.5 per cent. at 3 months old to 83 per cent. at 11 months old; the greatest rate of rise occurs between 5 and 7 months and afterwards slows down somewhat. With this rise in carcass percentage the proportions of pluck and alimentary canal etc. fall rapidly between 3 and 7 months and afterwards more slowly; the alimentary canal etc. ("unaccounted for") on the whole falling more than the pluck, which is to be expected since the latter includes the caul fat. Semmler (13) found in pigs that the lungs of young animals are relatively larger than those of old pigs of the same breed.

The actual rate of growth of the carcass, pluck and alimentary canal in lbs. per week is shown in Fig. 2 and also in Table VIII. From this

table it will be seen that 100 lbs. of growth in live weight from birth to 3 months consists of 74.3 lbs. carcase, 5.8 lbs. pluck and 19.9 lbs. alimentary canal, etc. whereas the same weight put on between 9 and 11 months consists of 94.5 lbs. carcase, 3.2 lbs. pluck and only 2.3 lbs. alimentary canal, etc.

This difference in the relative composition of the increase between animals of young and old ages should not be allowed to detract from the relative economy of meat production at early ages in general, but points to the conclusion that where young animals are being killed for meat it is very essential to use an early maturing breed in which the age changes are hastened.

Data have been collected from various authorities on the Carcase percentage of pigs of different live weights and are given in the table below:

	Live weight—lbs.					
	Birth—50	50—100	100—200	200—300	300—400	400—500
Robison(44)	—	77.7	83.4	87.6	88.2	88.2
Coburn(45)	—	72	74	78	80	87
Smith(46)	74.5	—	76	79	80	81
Tomhave(47)	—	—	80	84	—	—

It will be seen that there is a considerable divergence of opinion as to the dressed carcase percentage at different weights, which may be accounted for by differences in breeds and modes of dressing, but all agree that it increases with the rise in live weight. Whether this increase is a function of the age or of the live weight should be made clear. Henseler(48) has shown that it is due to the latter; he took two pigs from the same litter—one was fed well and the other starved, both being killed at the same age, but at very different live weights. The well-fed pig gave a carcase percentage of 87 per cent. and the starved one only 73 per cent. Under 'Correlation' (below) it is shown that the carcase percentage within any one breed increases with size independent of age.

That the carcase percentage will increase with age if the size is kept constant (by controlled feeding) would appear doubtful, since increased carcase percentage is brought about by the growth of muscles, fat and bones which attain their maximum rate of development late in life. When variations due to conformation and differential rates of growth of tissues in the different breeds are considered however it will be seen from Table VII that the carcase percentage may be independent of size: for example, Large Blacks of 5 months old weigh some 20 lbs. heavier than Middle Whites, but while the former have a carcase percentage of 74 per cent. the latter kill at 77 per cent.

406 *Growth and Development of Breeds and Crosses of Pigs*

Early Maturity. In order to obtain an estimate of the rate of maturity of an animal it is necessary to consider three factors. In the *first place* maturity so far as live weight is concerned is the rate at which an animal attains its mature weight. This factor is seen in Table IX for the various breeds, the weights at different ages being shown as a percentage of the weight at 11 months. This table shows that the Middle White is the most early maturing breed with the Berkshire following closely behind it. Figures for the other breeds are somewhat fragmentary but indicate that the Large White and Tamworth come next while the Lincolnshire Curly Coated and Large Black are last in this respect.

The *second* factor by which the rate of maturity may be measured is that of the carcass percentage at different ages. A breed which matures early forms bone, muscle, and fat early, and so the proportion of the carcass rises, that is, the age changes of low to high carcass percentage are hastened. Müller (6) believes that the changes causing early maturity are controlled by the glands of internal secretion. Fig. 4 shows the carcass percentages of the breeds at different ages (see also Table VII). At 3 and 5 months old the Berkshire has the highest carcass percentage followed by the Middle White, Large White and Large Black in the order named. At 7 and 9 months the Middle White overtakes the Berkshire, but the other breeds remain in the same relative order, although they tend more nearly to approach one another.

Another method of estimating the rate of maturity of different parts of the body is shown in Table X; the weights of parts at different ages are shown as a percentage of the weights of the parts at 11 months old. Considering the averages of the four breeds, whereas at 3 months old the alimentary canal etc. has made 10 per cent. of its ultimate growth the carcass has made only 23 per cent., the pluck 31 per cent. and the body as a whole 27 per cent.

The two breeds in which the figures are significant (Berkshire and Large White) stand in the same relative order as given above. If the Live Weight figures in this table for "Carcass Classes" are compared with those given in Table IX for the "Live Classes" it will be seen that the ratio is higher in the Carcass Classes; the reason has been pointed out above, namely, that the Carcass Class animals exhibited at the older ages are not so fat (and heavy) as those of the Live Classes and so the early maturity ratio is higher. For the same reason the ratio for Tamworths at 9 months in Table IX is probably rather high on account of the relatively small amount of fat this breed puts on at the older ages.

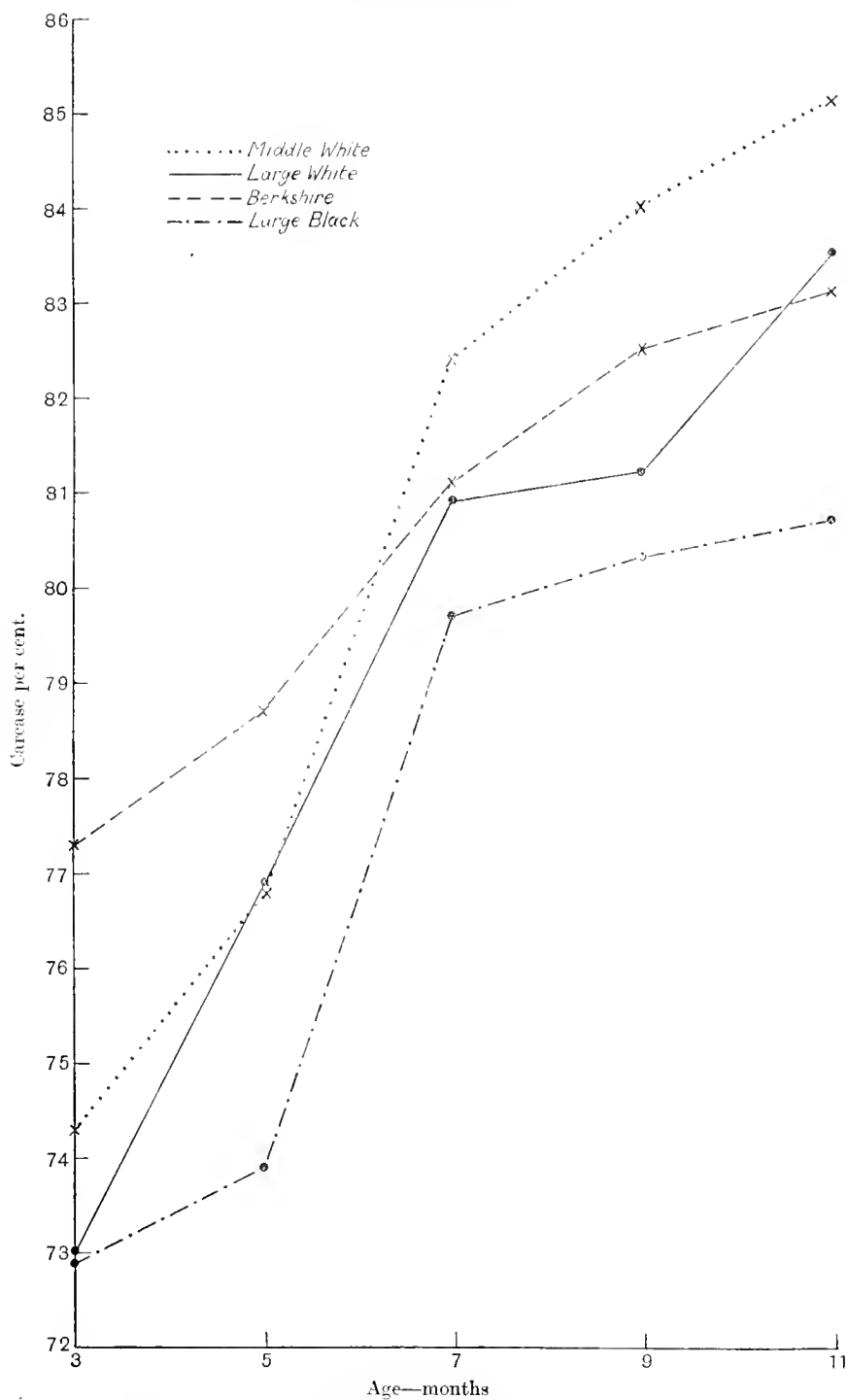


Fig. 4. Carcase percentage of various breeds of pigs at different ages.

408 *Growth and Development of Breeds and Crosses of Pigs*

Table IX. *Rate of maturity of different breeds of pigs—weight as percentage of weight at 11 months old.*

Age in months	...	Pen of 2 classes				Single pig classes
		3	5	7	9	9
Middle White	...	20	31	55	85	86
Berkshire	...	21	29	54	84	81
Large White	...	18	22	—	81	81
Tamworth	...	—	—	72	82	86
Large Black	...	—	—	65	79	—
Somersetshire	...	—	32	—	80	—
Lincolnshire Curly Coated	...	11	—	67	79	96
Dorset	...	—	—	—	—	68
Small White	...	—	—	55	79	—

Table X. *Rate of maturity of the body and its parts in different breeds of pigs—as percentage of the weight at 11 months old.*

Age in months—	3				5			
	Live	Carcase	Pluck	In- testines, etc.	Live	Carcase	Pluck	In- testines, etc.
Berkshire	29	27	36	40	41	39	51	52
Large White	21	18	30	35	38	35	51	54
Large Black	31	28	36	46	43	39	50	60
Middle White	21	18	33	38	33	30	45	55
Average	27	23	34	40	39	36	49	55

Age in months—	7				9			
	Live	Carcase	Pluck	In- testines, etc.	Live	Carcase	Pluck	In- testines, etc.
Berkshire	70	69	75	80	93	92	95	97
Large White	71	68	83	82	80	78	89	91
Large Black	83	82	79	91	94	93	88	98
Middle White	68	66	79	82	100	98	103	110
Average	73	71	79	84	92	90	94	99

The *third factor* in early maturity—the proportions of fat and muscle to bone in the carcase, it was not possible to determine from the data given in the records of the Show. This point remains to be decided by future investigation. It has long been known (Varlo(50)) that “no animal before it comes to the meridian of age or growth designed by nature will feed or lay on any quantity of fat.” That this fact is intimately connected with the rise in carcase percentage due to age changes however is now only in the process of being worked out. Lawes and Gilbert(51) showed that the state of fatness affected the carcase percentage and Mackenzie and Marshall(52) have shown that this also applies to the fat contained within the muscle itself which increases with the carcase percentage. Bormann(53)

found that early maturity was associated with an excessive amount of fat in pigs. Lucas(51) states that early maturity is associated with enlargement of the trunk together with the attached musculature and with a reduction in size of the extremities. Sanson(55) found that in early maturing animals the growth of the bones in length is arrested by early ossification of the epiphyses—another example of the hastening of age changes in early maturity. Nathusius(56) concluded from an examination of the skulls of a variety of pigs that in early maturing animals the skull is relatively broad and deep but with late maturing long and narrow like the wild boar, and that these proportions can be affected by nutrition during development. His results have been confirmed by Nehring(57). That early maturity can be hastened by feeding is well shown in Henseler's experiments(58) in comparing the composition of a starved pig with a well-fed one of the same age, the latter not only showing a larger live weight for age but also a higher carcass percentage. Varot and Fleniaux(59) found that in children the method of feeding affects the weight (muscle and fat development) but not the growth in height (bone development).

Cross-breeding. Table XI shows the average weights and ages of all cross-bred pigs shown during the period 1901–13. These weights have been translated into rates of growth per week and are given in Table XII. As differences in age make it impossible to compare the crosses with averages for pure breeds the weights of each cross have been calculated to common ages and in Table XIII are shown in comparison with the mean weight between the two breeds crossed; it is to this table that reference is made below.

In several cases the cross is larger than the heavier of the parent breeds—the Berkshire-Middle White and Berkshire-Tamworth crosses are outstanding examples—the same thing occurs in the majority of cases in the Berkshire-Large White cross. In many instances where the difference in weight between the breeds crossed is large, the cross although not actually heavier than the largest parent is heavier than the mean of the parent breeds. In only one case—the Berkshire-Lincolnshire Curly Coated cross—is the offspring consistently smaller than the mean of the parent breeds. Even if large size is dominant in some cases (although in the majority of animals the first cross is intermediate in size) there is in addition a large increase in size and vigour in the first cross. Whether this is due to additional size factors being brought in as Punnett and Bailey(60) found with fowls or to the recombination of characters eliminated by in-breeding remains to be proven. There is much accumulated evidence

to show (61) that pigs are particularly susceptible to in-breeding and consequently respond readily to an out-cross. The application of this principle has been made in Denmark where two pure breeds are kept for the purpose of crossing to produce the commercial pigs.

The reciprocal cross Berkshire-Large White is interesting because where the Berkshire sow is used the offspring at the earlier ages appear to be smaller than the reciprocal cross although they eventually reach the same size. Loudon (62) states that the way of stock improvement is best if the females are large and the males of smaller size; the female is then able to nourish the young better. It may be that Berkshires are not particularly good mothers and that pigs suckled by them do not grow so fast at first, but it is more probable that it is a case of sex linked inheritance of early maturity. This appears to be a case similar to that of the Aberdeen-Angus-Shorthorn cross in cattle (63) where early maturity seems to be inherited through the male rather than the female. Berkshire crosses with both Tamworths and Lincolnshire Curly Coated seem to behave in the same way; although no difference is seen when early maturity comes in from both sides such as in the Berkshire-Middle White cross.

Table XIV shows the rate of maturity of the crosses as a percentage of the weight at 11 months old and from it the facts mentioned above will be clearly seen. It will also be noticed that the general tendency seems to be not only for cross-breeding to increase the actual weight but also for it to increase the rate of maturity in live weight. Punnett and Bailey (64) found in rabbits that crossing distinct breeds may in some cases lead to early maturity in the first cross.

The comparative carcase weights of cross-bred pigs at different ages have been calculated by correcting for age and are shown in Table XV, as with the pure breeds the live weights are much below those of the live classes more particularly at the older ages.

Table XVI shows the proportions of the carcase, pluck and alimentary canal etc. to live weight in cross-bred pigs and when comparison is made with the pure breeds in Table VII it will be seen that, on the whole, there is a tendency for early maturity in carcase weight to be improved by cross-breeding when an early maturing is crossed with a late maturing breed (Berkshire-Large Black) or even when two late maturing ones (Large White-Large Black) are crossed together. When, however, two early maturing breeds (Middle White-Berkshire) are crossed no improvement in this respect is evident. The number on which these conclusions are based is small however, and they are at the best only tentative ones.

414 *Growth and Development of Breeds and Crosses of Pigs*

Wellmann (65) has published results of comparative carcass tests made with the Lincoln and Mangalitzia breeds and the crosses between them. He found that the live weight and carcass percentage of the cross-breeds at 14 months was higher than that of Mangalitzia's at 26 months old.

Table XIV. *Rate of maturity in live weight of cross-bred pigs—as percentage of weight at 11 months old.*

Age in months	Pen of 2 classes				Single pig classes
				3	5	7	9	
♂	♀							
{ Berkshire × Middle White	17	29	—	86	82
{ Middle White × Berkshire	22	32	51	85	—
{ Berkshire × Large White	25	30	—	92	89
{ Large White × Berkshire	21	22	—	79	78
{ Large White × Large Black	—	23	—	—	—
{ Large Black × Large White	—	22	61	85	—
{ Lincolnshire Curly Coated × Berkshire	—	—	—	73	—
{ Middle White × Large White	26	—	—	—	—

Selection. In order to study the progress made by breeders in altering the weights of pigs the records of the Show have been grouped in periods—the first from 1901 to 1906 both inclusive and the second from 1907 to 1913 both inclusive. The differences between the average weights of the two periods (Table XVII) would show the progress made.

Whenever the numbers exhibited are sufficient to be reliable they show an increase in weight. In one or two cases, as at the younger ages of 5 and 7 months, there has been a decrease but the numbers from which the results have been calculated are small.

The Large Black has shown the most notable increase in weight (Fig. 5) and it will be seen that the distribution curve has been shifted forward. This breed at 11 months old has made an average increase of 70 lbs. between the two periods; the Large White follows next with a rise of 50 lbs., the Middle White with 30 lbs. increase and lastly the Berkshire with about 5 lbs. increase. Whether this effect is due to further fattening or is actual growth in meat it is difficult to say as no figures are available to give an estimate of the state of fatness of the pigs shown. However, as the increase is only at 9 and 11 months and not at 5 or 7 months there is a strong indication that it is due to state of fatness.

In the Carcass Classes the Berkshire is the only breed which has been exhibited in sufficient numbers to make a comparison between the two periods possible and the data for this breed is shown in Table XVIII. At all ages, with the exception of 9 months old there has been a decrease in the carcass percentage and an increase in the proportion of the

Table XV. *Comparative carcase weights of cross-bred pigs.*

Age in months	3			5			7			Intes- tines, etc.					
	♂	♀	No.	Live	Car- case	Intes- tines, etc.	No.	Live	Car- case	Pluck	Intes- tines, etc.	Car- case	Pluck	Intes- tines, etc.			
			1	93.0	72.0	5.5	15.5	3	85.0	64.0	4.8	16.2	4	188.5	152.7	8.6	27.2
			—	—	—	—	—	1	109.3	83.6	5.3	20.4	—	—	—	—	—
			2	71.5	53.0	5.5	13.0	—	—	—	—	—	1	211.1	158.7	8.7	43.7
			1	70.0	47.0	4.0	19.0	1	150.1	128.2	8.2	22.7	2	187.7	119.8	8.3	29.6
			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
			—	—	—	—	—	1	105.0	84.0	7.0	14.0	—	—	—	—	—
			1	57.6	40.6	3.6	13.1	1	146.7	114.3	9.5	22.9	—	—	—	—	—
			—	—	—	—	—	—	—	—	—	—	2	250.0	205.0	12.2	32.8
			—	—	—	—	—	1	166.6	137.3	7.3	22.0	2	194.9	160.2	8.5	26.2
			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
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			—	—	—	—											

Table XVI. *Proportions of different organs and tissues in cross-bred pigs—as percentage of live weight.*

Age in months	3			5			7			9			11			
			Car- case	Pluck	Intes- tines, etc.	Car- case	Pluck	Intes- tines, etc.	Car- case	Pluck	Intes- tines, etc.	Car- case	Pluck	Intes- tines, etc.	Car- case	Pluck	Intes- tines, etc.	
5																		
	{ Middle White × Berkshire			77.4	5.9	16.7	75.3	5.6	19.4	81.0	4.6	14.4	83.8	4.1	12.1	83.9	4.5	11.6
	{ Berkshire × Middle White			—	—	—	76.5	4.8	18.7	—	—	—	—	—	—	85.5	4.1	10.4
	{ Large White × Berkshire			—	—	—	—	—	—	—	—	—	83.5	3.7	12.8	—	—	—
	{ Berkshire × Large White			74.1	7.7	18.2	—	—	—	74.8	3.7	21.5	86.0	4.1	9.6	—	—	—
	{ Tamworth × Berkshire			67.1	5.7	27.2	80.6	5.1	14.3	79.8	4.4	15.8	80.3	4.9	14.8	82.9	4.4	12.7
	{ Berkshire × Tamworth			—	—	—	—	—	—	—	—	—	83.1	5.2	11.7	82.5	4.7	12.8
	{ Large Black × Berkshire			—	—	—	80.0	6.7	13.3	—	—	—	—	—	—	—	—	—
	{ Berkshire × Large Black			—	—	—	—	—	—	—	—	—	84.8	4.2	14.0	—	—	—
	{ Large White × Large Black			76.7	6.3	17.0	77.9	6.5	15.6	—	—	—	—	—	—	—	—	—
	{ Large Black × Large White			—	—	—	—	—	—	82.0	4.9	13.1	83.3	4.3	12.4	—	—	—
	{ Large White × Middle White			—	—	—	82.4	4.4	13.2	82.2	4.4	13.4	82.2	4.7	13.1	—	—	—

alimentary canal. It is difficult to give an explanation of this fact unless it be due to changes in the systems of management and amount of exercise given. The same thing has occurred in sheep (66).

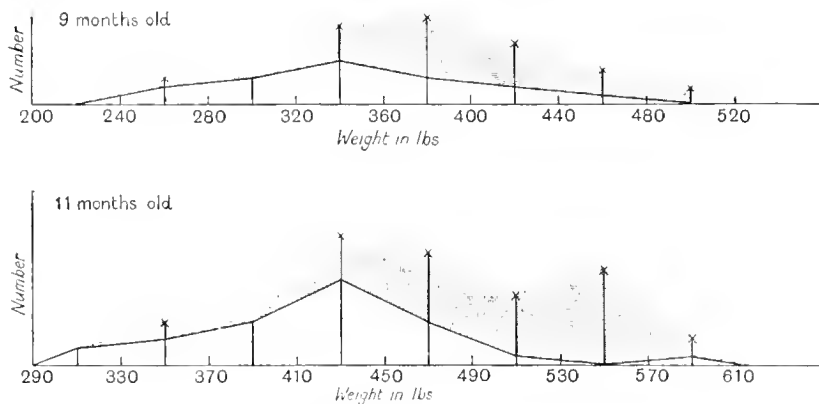


Fig. 5. Distribution curves of live weight in Large Black pigs.

Light curve—1901-6. Shaded curve—1907-13.

Table XVII. *Changes in live weights of pigs from Period I (1901-6) to Period II (1907-13).*

		Weight in lbs.				
	Period	3 months	5 months	7 months	9 months	11 months
Middle White	I	—	125	236	324	369
	II	77	109	195	327	398
Large White	I	—	161	—	359	447
	II	86	105	—	395	499
Large Black	I	—	—	292	350	427
	II	—	—	354	399	497
Berkshire	I	—	148	253	333	399
	II	85	107	195	342	405

Numbers of pigs from which results calculated.

Middle White	I	—	2	6	76	62
	II	10	60	14	68	66
Large White	I	—	4	—	38	50
	II	4	34	—	70	64
Large Black	I	—	—	20	34	56
	II	—	—	8	44	66
Berkshire	I	—	8	10	128	120
	II	16	124	22	118	148

Table XVIII. *Changes in the proportional development of Berkshire pigs from Period I (1903-6) to Period II (1907-13).*

Period	Percentage of live weight														
	3 months			5 months			7 months			9 months			11 months		
	Carcass	Pluck	Intestines, etc.	Carcass	Pluck	Intestines, etc.	Carcass	Pluck	Intestines, etc.	Carcass	Pluck	Intestines, etc.	Carcass	Pluck	Intestines, etc.
I	67.1	6.5	26.1	78.2	5.3	16.5	82.1	4.7	13.2	82.7	4.5	12.8	83.7	4.5	11.8
II	77.3	5.3	17.4	77.6	5.5	16.9	80.7	4.8	14.5	83.1	4.5	12.4	82.8	4.3	12.9
	Numbers from which calculated														
I	1			23			9			18			11		
II	26			35			23			51			27		

Individual variation. All the figures given in this paper are averages of a number of pigs and there is a large amount of individual variation in weight apart from those due to causes which are known. Such things as strain, state of fatness, etc., influence the live weight and all these come under the heading of individual variation. One of the most important causes of variation is the amount of food contained in the alimentary canal; while it is not so important as in cattle and sheep where the contained food may be as much as 14 per cent. of the live weight yet it is a large factor. Table B shows that it may vary from 2 to 11 per cent. of the live weight; while not all of this can be eliminated yet it is sufficient to cause considerable variation.

As a measure of this variation in the various breeds and at different ages the standard deviation has been calculated and is shown in Table XIX; the coefficient of variability has been used as the measure of variation. The Large Black breed shows greater variation than the others and this is probably in part due to the increases in weight which have been made in this breed in the last few years (see Selection above). It is notable that the first crosses show rather less variation than the pure breeds.

On the average of all breeds the coefficient of variability increases with age up to 7 months and then decreases again, variability being greatest when the rate of growth is at its maximum. This will be seen if the coefficients of variability at the base of Table XIX are compared with the rates of growth at the different ages given in Fig. 2. There is, however, more variation at the younger ages than at the older ones, as might be expected if improvements both by feeding and breeding were being made in the rate of early maturity. That variability is greatest when the rate of increase is at its maximum was first pointed out by King (67) for rats. Robertson's (68) results also show it to be true for mice

and men. Drummond *et al.* (69) have found that the growth of the pig is affected by lack of vitamins in the diet while Elliot, Crichton and Orr (70) have shown that the more rapid growing pigs are the quickest to be affected by rickets.

Table XIX. *Variation in live weight of pigs* (pens of 2).

		<i>Standard deviation, lbs.</i>				
Age in months	...	3	5	7	9	11
Middle White	...	8.2	17.5	53.7	35.4	43.1
Large White	...	7.6	22.5	—	47.5	57.4
Large Black	...	—	—	49.5	58.1	67.2
Berkshire	...	11.6	16.4	76.5	32.0	43.3
Middle White × Berkshire	...	7.3	13.2	87.6	40.9	28.9
Berkshire × Middle White	...	11.2	16.6	—	25.4	42.2

Coefficient of variability.

Middle White107	.148	.256	.109	.112
Large White088	.210	—	.122	.119
Large Black	...	—	—	.162	.157	.143
Berkshire141	.141	.351	.095	.108
Middle White × Berkshire080	.099	.387	.115	.069
Berkshire × Middle White149	.130	—	.068	.097
Average (5 breeds)113	.146	.331	.102	.101

Table XX. *Variation in proportional development of Berkshire pigs.*

Age in months	...	3			5		
		Carcase	Pluck	Intestines, etc.	Carcase	Pluck	Intestines, etc.
Standard deviation—lbs.		2.70	0.80	2.70	3.13	0.68	3.05
Coefficient of variability		.035	.148	.151	.040	.124	.178
Age in months	...	7			9		
		Carcase	Pluck	Intestines, etc.	Carcase	Pluck	Intestines, etc.
Standard deviation—lbs.		3.39	0.51	3.04	2.08	0.39	2.04
Coefficient of variability		.042	.106	.208	.025	.087	.155
Age in months	...	11					
		Carcase	Pluck	Intestines, etc.			
Standard deviation—lbs.		2.09	0.45	1.98			
Coefficient of variability		.025	.102	.155			

The standard deviation and coefficient of variability of the proportional development of the parts of the body in the Berkshire breed at different ages are shown in Table XX. The carcase percentage like the live weight increases in variability up to 7 months and then decreases again, the variability being greatest at the period when the change in carcase percentage is most rapid (see Fig. 3). The percentage of pluck, on the other hand, steadily decreases in variability from 3 to 9 months; this

is contrary to what occurs in sheep where variability in pluck increases with age but it should be remembered that with pigs the pluck includes the caul fat and that this in sheep decreases in variability with age. The variability in the proportion of alimentary canal etc. undergoes the same changes as the carcass.

The alimentary canal has the greatest coefficient of variability, the pluck (including caul fat) comes next and the carcass has least. The causes underlying the variation in carcass percentage at any one age are probably mainly due to differences in fatness, early maturity and the amount of food contained in the alimentary canal; other factors however play a part. Sanborn(71) and Henry(72) found that a ration low in protein affected the proportions of the body, and their results have been confirmed by Emmett *et al.*(73). Hart and McCollum(74) have shown that a diet of maize alone reduced the rate of growth in pigs considerably while Jackson and Stewart(75) have shown that rats subjected to periods of deficiency in diet were subnormal as regards skeletal and muscular development, while the viscera were above normal in weight. Elliot, Crichton and Orr(76) found that in pigs with rickets the head was abnormally large and Henseler(76) found that by controlling the nutrition of pigs he could not only affect the live weight but also the proportions of the body.

Correlation. In order to determine the effect of weight, independent of age, on the proportions of the body and also the correlation existing between the proportions of the different parts of the body Table XXI has been prepared. This table shows that, independent of age, a high live weight is correlated with a large percentage of carcass and a small percentage of pluck and alimentary canal, etc.; that is within a breed the heavier a pig is for its age the higher will be the proportion of carcass to live weight. This fact is consistently confirmed by the various groupings made. The remainder of the table shows that the proportions of the carcass vary inversely as the proportions of the pluck and alimentary canal, this appearing as the result of every grouping.

Similar results were obtained experimentally by Henseler(76) who took two pigs from the same litter, one was well fed and the other starved; both were killed at 9 months old. The starved one weighed 58 lbs. and the well fed one 279 lbs., the former having a carcass percentage of 73 per cent., while the latter killed at 87 per cent.

The main cause of the increased carcass percentage with age is the development of muscle and fat rather than great alterations in the size of the other organs of the body.

The above conclusions seem to point to the fact that a large animal is a more economical producer of meat than a small one of the same age, especially when considered in conjunction with Voit's (77) results which confirmed Rubner's theory that the amount of energy required by a young animal when calculated in relation to unit of body surface is the same as that of an adult, the younger and smaller animals having a larger surface per kilo body weight than adult and larger animals.

Table XXI. *Correlation of live weight and parts of the body in Berkshire pigs.*

Grouped by	Group	Live weight lbs.	Carcase %	Pluck %	Intestines, etc. %
Live weight	High	—	81.3	4.7	14.0
	Average	—	80.1	4.9	15.0
	Low	—	78.4	5.2	16.4
% carcass	High	189.4	—	4.6	12.6
	Average	166.2	—	4.9	14.9
	Low	150.7	—	5.3	17.5
% pluck	High	149.5	78.4	—	16.0
	Average	168.6	80.1	—	15.0
	Low	187.9	81.3	—	14.4
% intestines, etc.	High	151.2	77.0	5.0	—
	Average	167.9	80.2	4.9	—
	Low	186.5	82.6	4.8	—

My thanks are due to Mr E. J. Powell, Secretary of the Smithfield Club, who has very kindly supplied me with records of the Shows, to Mr Charles Bone of the Central Meat Markets, London, who has given me information on the methods of slaughter and dressing adopted, and lastly to Dr F. H. A. Marshall, F.R.S. who has given me the benefit of his advice in the preparation of this paper.

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422 *Growth and Development of Breeds and Crosses of Pigs*

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